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A transient pool of nuclear F-actin at mitotic exit controls chromatin organization.

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Reestablishment of nuclear structure and chromatin organization after cell division is integral for genome regulation or development and is frequently altered during cancer progression. The mechanisms underlying chromatin expansion in daughter cells remain largely unclear. Here, we describe the transient formation of nuclear actin filaments (F-actin) during mitotic exit. These nuclear F-actin structures assemble in daughter cell nuclei and undergo dynamic reorganization to promote nuclear protrusions and volume expansion throughout early G₁ of the cell cycle. Specific inhibition of this nuclear F-actin assembly impaired nuclear expansion and chromatin decondensation after mitosis and during early mouse embryonic development. Biochemical screening for mitotic nuclear F-actin interactors identified the actin-disassembling factor Cofilin-1. Optogenetic regulation of Cofilin-1 revealed its critical role for controlling timing, turnover and dynamics of F-actin assembly inside daughter cell nuclei. Our findings identify a cell cycle-specific and spatiotemporally controlled form of nuclear F-actin that reorganizes the mammalian nucleus after mitosis.

Introduction

Cytoplasmic actin polymerization at plasma membranes is an essential and versatile process that defines cellular shape, determines cell polarity, cell-cell and cell-matrix interactions, and drives cytokinesis¹. In addition, it is well established that actin resides in the nuclear compartment of somatic cells^{2,3}. For example, monomeric actin is stably assembled into

chromatin remodeling complexes ^{4,5}, while a dynamic pool of actin appears to constantly shuttle between the nucleus and cytoplasm in an Importin 9- and Exportin 6-dependent manner ⁶. Similarly, many actin-regulatory proteins have been described to enter the nuclear compartment ². More recently, using nuclear-targeted probes, the presence of F-actin structures was demonstrated in mammalian cell nuclei in response to serum, integrin signaling or DNA damage ⁷⁻⁹. However, whether transient and dynamic nuclear actin filaments exert fundamental structural functions in somatic cells to spatially reorganize nuclear architecture has not been investigated ¹⁰.

Mitotic cell division relies on a complex cascade of mechanistic processes to precisely ensure maintenance of genomic organization and integrity in the emerging daughter cells. During mitotic exit, newly formed cells undergo a profound reorganization of their nuclear content to reestablish an interphase nucleus, which is accompanied by a striking expansion in nuclear size and volume ^{11,12}. Further key processes include the reformation of a nuclear envelope and lamina, assembly of nuclear pore complexes, and decondensation as well as reorganization of the highly condensed mitotic chromosomes ^{13,14}. Surprisingly, the cellular mechanisms, which drive nuclear expansion while reversing mitotic chromosomes into an interphase chromatin state, remain largely unexplored ^{11,15,16}.

RESULTS

Transient nuclear F-actin assembly during mitotic exit

We recently reported an approach to monitor endogenous nuclear F-actin dynamics without detectable effects on nucleocytoplasmic shuttling or the polymerization state of actin⁸. Our live-cell compatible approach relies on a transiently binding nanobody directed against actin fused to a nuclear localization signal (anti-Actin-Chromobody-GFP-NLS), herein referred to as nAC-GFP (nuclear Actin-Chromobody-GFP)⁸. While examining mouse fibroblasts stably expressing nAC-GFP together with LifeAct-mCherry to co-visualize cytoplasmic actin, we observed the striking and transient appearance of nuclear actin filament structures when daughter cell nuclei formed (Fig. 1a and Supplementary Video 1). These actin filaments were constantly and dynamically reorganized within the nuclear compartment (as visualized by a nanobody against Lamin A/C), arguing for a spatiotemporal function during the final stages of cell division (Fig. 1b and Supplementary Video 2). Of note, nuclear actin concentrations appeared to be constant during exit from mitosis and were not affected by the presence of nAC (Supplementary Fig. 1a, b).

Cell cycle-associated nuclear actin filaments could be detected and quantified using nAC-GFP or a shuttling Actin-Chromobody (sAC) with comparable frequencies (Fig. 1c, 3a and Supplementary Video 3). Transient nuclear actin polymerization persisted for 60-70 minutes during early G₁ phase, followed by filament disassembly upon further progression into interphase (Fig. 1d and Supplementary Video 1). Importantly, we confirmed our observations by using the F-actin marker phalloidin in fixed, but otherwise native, untreated cells (Fig. 1e). Nuclear actin polymerization at mitotic exit could also be observed in MCF10A breast epithelial, RPE-1 retinal pigmented epithelial as well as HT1080 fibrosarcoma cells, arguing for a conserved feature among mammalian cell types (Supplementary Fig. 1c-e).

Mitotic nuclear actin filaments were not affected by silencing of the nucleoskeletal proteins Emerin or Lamin A/C (Supplementary Fig. 1f-h), or expression of a dominant-negative KASH (Klarsicht/ANC-1/Syne-1 homology) domain (Supplementary Fig. 1i), shown to disrupt the LINC (Linker of Nucleoskeleton and Cytoskeleton) complex¹⁷, suggesting that these filaments are different in origin and function from those forming upon cell spreading and integrin-dependent signaling⁸.

Analysis of nuclear F-actin at mitotic exit using super-resolution microscopy

Next, cells stably expressing nAC fused to the photoconvertible fluorescent protein Dendra2 were imaged by PALM (Fig. 2a and Supplementary Fig. 2a). Under these conditions, 96% of all cells showed nuclear actin filaments at mitotic exit. Actin filaments were of several micrometer lengths with an apparent width of about 50-90 nm, which can be explained by single actin fibers stained with nAC-Dendra2 of about 10 nm width and thin bundles of about 2-5 fiber diameters convolved with the achieved PALM resolution of about 40 nm (Fig. 2b and Supplementary Fig. 2a, b).

To further investigate native cells, phalloidin staining was performed to confirm these findings using STORM imaging on cells fixed at defined time points during mitotic exit (Supplementary Fig. 2c). This revealed nuclear actin fibers for up to 60 minutes after anaphase. The reduced background and improved resolution of 30 nm allowed us to detect a population of even thinner, 40 nm wide nuclear actin filaments in addition to thicker, more bundled subsets of F-actin structures (Fig. 2c, d and Supplementary Fig. 2c).

104

105 **Postmitotic nuclear volume expansion requires nuclear actin polymerization**

106 To gain insight into the functional role of nuclear F-actin formation in early G₁, we visualized
107 nuclear actin filaments together with a fluorescently-labeled histone H2B to label the nuclear
108 compartment. This revealed a substantial expansion of nuclear volume during the period of
109 nuclear F-actin assembly as well as distinct nuclear protrusions associated with the emerging
110 dynamic nuclear F-actin structures (Fig. 3a and Supplementary Video 4).

111 To assess a potential role of nuclear F-actin formation in reshaping the newly forming
112 nuclei after cell division, we first tested for the effects of various pharmacological inhibitors of
113 the actin cytoskeleton that were directly added under the microscope to cells exiting mitosis.
114 The actin-depolymerizing agents Cytochalasin D or Latrunculin B robustly impaired nuclear
115 volume expansion, while the Arp2/3 inhibitor CK-666 had no effect (Supplementary Fig. 3a, b).

116 As pharmacological agents interfere globally with actin dynamics, we aimed to directly
117 assess the impact of nuclear actin by expression of its specific nuclear export factor Exportin 6
118 to enhance nuclear export of actin monomers (Supplementary Fig. 1b)¹⁵. This not only resulted
119 in a strongly reduced number of cells displaying nuclear F-actin during mitotic exit (Fig. 3b), but
120 also in a significantly impaired volume expansion of daughter nuclei (Fig. 3c, d).

121 To more specifically address the role of polymerized nuclear actin, we generated cell
122 lines stably expressing a doxycycline-inducible, nuclear-targeted version of the polymerization-
123 deficient actin mutant actin^{R62D} (NLS-BFP-actin^{R62D})^{8,18}, to dilute the amount of polymerization-
124 competent actin monomers within the nuclear compartment. Similar to expression of Exportin

6, this approach resulted in a strong reduction of nuclear actin filaments as well as impaired nuclear expansion compared to control cells expressing BFP-NLS only (Fig. 3e-g). To control for any effects potentially arising from an increased concentration of nuclear actin monomers, we additionally compared the effects of actin^{R62D} to wild-type actin (actin^{wt}) instead of BFP. Given the potential limitations of fluorophore-tagged actin¹⁹, we generated doxycycline-inducible Flag-NLS-actin constructs fused to a self-cleavable SNAP-tag, which allowed us to indirectly identify expression of these Flag-actin derivatives in live dividing cells (Supplementary Fig. 3c-f). Importantly, this experimental setup confirmed our results (Fig. 3g); clearly demonstrating the critical need of a polymerization-competent pool of nuclear actin required for nuclear volume expansion.

Noteworthy, the effects of nuclear F-actin on nuclear volume expansion were specific for cells at the mitotic exit, as no differences in nuclear volume could be detected among nuclei of cells arrested in interphase during induction of actin^{R62D} expression (Supplementary Fig. 3g). Furthermore, global transcriptional inhibition by the CDK inhibitory drug Flavopiridol during cell division did not affect the rate of daughter nuclei expansion (Supplementary Fig. 3h, i), excluding altered transcription as a cause of the observed phenotype.

Using atomic force microscopy (AFM) on isolated nuclei from synchronized live cells enabled us to visualize the structure of the nuclear surface in early G₁ as well as interphase (Fig. 3h). This revealed a remarkably rough nuclear surface indicative of nuclear protrusions in postmitotic control cells expressing Flag-NLS-actin^{wt}, which were absent in Flag-NLS-actin^{R62D} expressing nuclei displaying an overall much flatter nuclear surface (Fig. 3h, i). Similar nuclear protrusions were observed on postmitotic nuclei from untransfected cells. Of note, induction of

chromatin decompaction by Trichostatin A (TSA)-treatment of interphase nuclei²⁰ did not result in an increased nuclear surface roughness (Fig. 3h, j), arguing for a cell cycle-specific phenomenon that critically depends on the involvement of nuclear F-actin formation in early G₁.

Nuclear actin assembly affects early G₁ chromatin reorganization

While visualizing nuclear actin dynamics together with the histone H2B, we found nuclear F-actin to reside within interchromatin spaces in the emerging daughter cell nuclei (Fig. 4a, Supplementary Video 3). This observation as well as the time period of nuclear actin polymerization during which chromatin decondensation takes place¹², prompted us to further investigate a potential role for F-actin in this process.

Quantifications of chromatin density assessed by 3D H2B-mCherry fluorescence intensities revealed a significantly higher degree of chromatin compaction in postmitotic nuclei impaired for the assembly of nuclear F-actin either by expression of Exportin 6 or nuclear-targeted actin^{R62D} (Fig. 4b-e).

To more directly measure the degree of chromatin compaction, we established a fluorescent lifetime imaging microscopy (FLIM) assay to determine fluorescence energy transfer (FRET) between GFP- and mCherry-tagged histone H2B (Supplementary Fig. 4a, b)²¹. Consistent with a role of nuclear F-actin in postmitotic chromatin dynamics, we found that induced expression of Flag-NLS-actin^{R62D} but not Flag-NLS-actin^{wt} (Fig. 4f, g), as well as expression of

Exportin 6 (Fig. 4h, i) resulted in a significantly reduced fluorescence lifetime of GFP-H2B in synchronized early G₁ cells (Fig. 4f-i).

Given the conserved roles of increased histone H3 Ser-10 phosphorylation (H3S10ph) and decreased histone H4 Lys-16 acetylation (H4K16ac) in mitotic chromatin condensation²², we confirmed the effects of altered nuclear F-actin assembly on chromatin reorganization by analyzing these histone modifications in cells synchronized by mitotic shake-off. While control cells (BFP-NLS) reestablished lower levels of H3S10ph and higher levels of H4K16ac within 90 minutes, cells expressing NLS-BFP-actin^{R62D} sustained mitotic levels of these histone modifications (Supplementary Fig. 4c). In addition, we found higher nuclear levels of Aurora B, a kinase responsible for H3S10 phosphorylation²³, as well as a lower levels of KAT5, known to mediate H4K16 acetylation²⁴, in cells expressing Flag-NLS-actin^{R62D} (Supplementary Fig. 4d, e). Consistently, chromatin obtained from cells exiting mitosis and expressing NLS-BFP-actin^{R62D} appeared more resistant to MNase digestion (Supplementary Fig. 4f), arguing for reduced chromatin accessibility.

Furthermore, electron microscopy of cryopreserved samples allowed us to directly quantify the amount of condensed chromatin, which appears as an electron-dense structure in unlabeled samples (Fig. 4j and Supplementary Fig. 4g). This approach confirmed a highly significant increase in the proportion of condensed chromatin at mitotic exit in cells expressing either nuclear-targeted actin^{R62D} (Fig. 4j, k) or Exportin 6 (Fig. 4l and Supplementary Fig. 4h) compared to control. Altogether, these data support a requirement for polymerization-competent nuclear actin to achieve proper chromatin organization after mitosis.

Nuclear F-actin is formed and required for nuclear volume expansion in early mouse embryos

To gain insight into the biological consequences of impaired mitotic nuclear actin assembly we determined general transcriptional activity by quantifying nuclear 3D RNA polymerase II phospho-Serine 5 (pS5) fluorescence. This revealed strongly reduced transcriptional activity in cells expressing GFP-Exportin 6 (Fig. 5a and Supplementary Fig. 5a) as well as significantly lower proliferation rates (Fig. 5b).

Next, we asked if similar functions of nuclear F-actin are exerted during development. For this, we investigated fertilized mouse embryos, which rely on substantial chromatin decondensation of sperm and oocyte nuclei. 150 ng mRNA of nAC-GFP was injected into mouse fertilized embryos and analyzed at indicated time points post insemination (hpi) (Fig. 5c). Notably, nuclear actin filaments were readily detected in pronuclei, as well as in the early G₁ phase after the first mitotic division (71.43%, 18 hpi) and disassembled upon further cell cycle progression of the 2-cell embryos (25%, 24 hpi) (Fig. 5d). Of note, expression of actin^{R62D}-HA-NLS or Exportin 6 inhibited nuclear volume expansion after the first mitotic division (Fig. 5e, f), consistent with our findings in mammalian somatic cells. Furthermore, a significant developmental delay was observed in Exportin 6 mRNA-injected embryos (Fig. 5g and Supplementary Fig. 5b), providing further evidence for a physiological role of nuclear F-actin assembly in the early steps of mouse embryo development.

Cofilin-1 controls nuclear F-actin assembly, volume expansion and chromatin organization in daughter cell nuclei

As siRNA against several known actin assembly factors had no obvious effect on nuclear F-actin formation (Supplementary Table 1) and to gain further mechanistic insight, we performed a proteomic screen using biotin-phalloidin to identify potential nuclear F-actin binding proteins from nuclear fractions of cells released from a mitotic nocodazole arrest (Fig. 6a, b). Hereby, we identified the F-actin disassembly factor Cofilin-1 as a candidate interactor (Fig. 6c, d and Supplementary Table 1). Since Cofilin-1 is inactivated by phosphorylation on Ser-3 (p-Cofilin)²⁵, we analyzed nuclear p-Cofilin levels by measuring 3D nuclear fluorescence intensities in synchronized NIH3T3 cells and observed a pronounced increase in nuclear p-Cofilin levels during mitotic exit, revealing Cofilin-1 as a cell cycle-controlled nuclear actin regulator (Fig. 6e and Supplementary Fig. 6a-d). Indeed, and consistent with the role of Cofilin-1 in F-actin disassembly, siRNA against Cofilin-1 resulted in a pronounced stabilization of nuclear F-actin during mitotic exit (Fig. 6f, g and Supplementary Video 5).

To specifically address the nuclear function of Cofilin-1 during this cell cycle phase, we generated cells expressing either wildtype (WT-Cofilin) or cytoplasm-targeted Cofilin-1 (NES-Cofilin; NES, nuclear export signal) resistant to siRNA targeting (Fig. 6h, i). Imaging mitotic cells revealed that upon silencing of endogenous Cofilin-1, cells devoid of its nuclear localization failed to prevent extensive nuclear F-actin stabilization during mitotic exit (Fig. 6j and Supplementary Fig. 6e) similar to depletion of total Cofilin-1 (Fig. 6g). Consistent with deregulated actin filament dynamics and turnover, this resulted in defective nuclear volume expansion (Fig. 6k) and chromatin decompaction (Fig. 6l), underscoring the critical importance of dynamic F-actin reorganization for the processes of nuclear volume expansion and chromatin reorganization.

Optogenetic control of Cofilin-1 establishes its role in reorganizing daughter cell nuclei after mitosis

To corroborate our findings that nuclear Cofilin-1 controls chromatin dynamics, we expressed a nuclear-targeted version of Cofilin-1 (NLS-Cofilin) to inhibit nuclear F-actin formation in early G₁ (Supplementary Fig. 6f), and observed a striking defect in establishing open chromatin in cryo-EM samples (Fig. 7a, b).

To directly and reversibly control Cofilin-1 function within the nucleus in real time, we generated an optogenetic Cofilin-1 (opto-Cofilin) (Fig. 7c) based on a previously described light-inducible nuclear export system²⁶. Under control conditions, opto-Cofilin exhibited a subcellular distribution similar to WT-cofilin, but allowed for rapid and efficient light-induced nuclear export within 200 seconds, which could be reverted within 500 seconds after illumination was switched off (Fig. 7d and Supplementary Video 6).

In cells silenced for endogenous Cofilin-1 (Supplementary Fig. 6g), light-induced nuclear export of opto-Cofilin resulted in extensive stabilization of nuclear F-actin during mitotic exit (Fig. 7e and Supplementary Videos 7, 8), while its timely controlled nuclear re-accumulation, by switching off illumination, triggered rapid re-organization and successive disassembly of nuclear F-actin (Fig. 7e and Supplementary Video 8). Notably, light-controlled export of opto-Cofilin during mitotic exit also resulted in arrested growth of daughter cell nuclei, while nuclear reimport of opto-Cofilin promoted their further volume expansion (Fig. 7f).

Together, these results uncover a critical nuclear-specific function of Cofilin-1 in spatiotemporally controlling actin dynamics for nuclear reorganization in the early phases after mitotic cell division.

DISCUSSION

Here, we discovered dynamic and transient F-actin assembly in the growing nuclei of daughter cells exiting mitosis. The mechanisms and cellular factors that determine nuclear volume regulation are poorly understood¹¹. Our data reveal a key function for nuclear actin filaments in nuclear volume and chromatin expansion during mitotic exit as well as a critical nuclear function of Cofilin-1 in tightly controlling the spatiotemporal turnover of these actin filaments. As such, nuclear reorganization during mitotic exit is impaired upon loss of polymerization-competent nuclear actin as well as excessive nuclear F-actin formation, illustrating that the dynamic interplay between polymerization and depolymerization of nuclear F-actin appears to be critical during this cell cycle-specific process. Accordingly, it is tempting to speculate that additional actin-regulatory factors as well as actin bundling proteins are involved in nuclear actin assembly during early G₁.

Since cytoskeletal actin dynamics are well known to exert contractile and mechanical forces in order to shape or move a variety of cellular components²⁷ and in light of our observation of F-actin-dependent nuclear protrusions, one may envisage similar functions for nuclear F-actin during mitotic exit in rearranging the chromatin and nuclear content of mammalian cells. Thus, future work will be directed to dissect whether the role for nuclear

actin in promoting efficient reorganization of chromatin is primarily exerted through direct effects of F-actin on chromatin, or more indirectly through expanding and reshaping the nuclear compartment, or both.

Our findings thus open a perspective to gain a better understanding of nuclear actin filament dynamics and its role in regulating spatiotemporal chromatin organization and maintenance of a defined nuclear architecture, all of which have profound implications for genome stability and regulation in health and disease.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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355

356 **Author Contributions:** C.B., M.P. and R.G. conceived the study. C.B., M.P., and A.S. performed
357 and analysed most of the experiments with help from R.G. and A.K.; R.H. assisted in electron
358 microscopy, D.A. in FLIM/FRET; K.Mo., S.M. and K.Mi. performed experiments on fertilized
359 mouse embryos; E.M.K, D.V. and U.E. performed PALM/STORM, and S.B. mass spectrometry.
360 R.G. and C.B. wrote the manuscript.

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362 www.nature.com/reprints. The authors declare no competing financial interests.
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Figure Legends

Figure 1 | Spatiotemporal features and dynamics of nuclear F-actin formation at mitotic exit.

(a) Time-lapse imaging of NIH3T3 cells stably expressing nAC-GFP (nuclear Actin-Chromobody-GFP, anti-Actin-Chromobody-GFP-NLS, green, grey in magnifications) together with LifeAct-mCherry (red) during anaphase and mitotic exit. The asterisk indicates a nucleus shown magnified in the lower panel. Images show maximum intensity projections of confocal z-stacks. See also Supplementary Video 1. Scale bar, 10 μm ; time stamp, h:min:s. **(b)** Live cell imaging of NIH3T3 cells stably expressing nAC-GFP (green) together with Lamin-Cb-SNAP (Lamin-Chromobody-SNAP, labelled by SiR-647, magenta) during mitotic exit. The nucleus indicated by an asterisk is shown magnified for individual time points. See also Supplementary Video 2. Scale bar, 10 μm (overview) and 1 μm (magnifications). **(c)** Quantification of incidence and **(d)** duration of nuclear actin filament formation at mitotic exit using indicated nuclear actin probes. Data are shown as mean + SEM from at least $n=3$ independent experiments and $n=60$ (nAC-GFP), $n=30$ (sAC-GFP, shuttling Actin-Chromobody, anti-Actin-Chromobody-GFP-NLS-NES), $n=60$ (phalloidin, formaldehyde fixation 70 min after mitotic shake-off) mitotic events. **(e)** NIH3T3 cells at mitotic exit were fixed using glutaraldehyde and stained for actin filaments using phalloidin-Atto 488. The area indicated by a dashed rectangle is shown magnified for individual confocal slices in z-direction with a step size of 0.37 μm . Scale bar, 10 μm (overview) and 1 μm (magnifications).

Figure 2 | Super-resolution microscopy reveals structural features of nuclear F-actin at mitotic exit.

(a) Photoactivated localization microscopy (PALM) of nuclear actin filaments in fixed NIH3T3 cells stably expressing nAC-Dendra2 (anti-Actin-Chromobody-Dendra2-NLS, orange) at mitotic exit. DIC (differential interference contrast) images were acquired over time to monitor mitotic progression and to allow for time-defined fixation 20 min after anaphase. Experimental resolution after drift correction is 33 ± 1 nm. Scale bars, 5 μ m. **(b)** Filament widths of nAC-Dendra2-labeled nuclear actin filaments ($n = 81$, data were collected from at least $n=3$ independent experiments) as histograms (up) with a bin size of 10.65 nm (x-axis) plotted against frequency (y-axis). Full data is represented underneath the histograms as box + scatter plots with the same x-axis. The box marks the first and third quartiles and the indent represents the median. Whiskers mark minimal and maximal values. **(c)** Stochastic Optical Reconstruction Microscopy (STORM) of phalloidin-stained nuclear actin filaments in native NIH3T3 cells at mitotic exit. The overview image focuses on a nucleus 45 min after anaphase (see also Supplementary Fig. 2c). The magnifications show representative thin (1) and thick (2) filaments with different labelling densities. Scale bars, 5 μ m (overview) and 200 nm (magnifications). **(d)** Filament widths of phalloidin-labelled nuclear actin filaments ($n=53$, data were collected from $n=1$ experiment) as histograms (up) with a bin size of 14.3 nm (x-axis) plotted against frequency (y-axis). Full data is represented underneath the histograms as box + scatter plots with the same x-axis. The box marks the first and third quartiles and the indent represents the median. Whiskers mark minimal and maximal values.

Figure 3 | Nuclear F-actin reshapes nuclei, and promotes nuclear volume expansion after mitotic cell division.

409 **(a)** NIH3T3 cells stably expressing nAC-GFP (green) together with H2B-mCherry (red) during
 410 mitotic exit. Asterisk indicates the nucleus shown for additional time points. Dashed rectangles
 411 indicate areas of dynamic nuclear protrusions, shown magnified over time. Arrows indicate
 412 direction of protrusions. See also Supplementary Video 4. Scale bar, 10 μm (overviews) and 1
 413 μm (magnifications); time stamp, min:s. **(b)** NIH3T3 cells expressing nAC-GFP (green) together
 414 with mCherry or mCherry-Exportin 6 (gray, insets) after anaphase. Figures give proportion of
 415 cells showing nuclear F-actin in the presence (n=15) or absence (n=17 mitotic events) of GFP-
 416 Exportin 6, visualized by nAC-mCherry. Scale bar, 10 μm . **(c)** Nuclear expansion after anaphase
 417 (00:00) was visualized by H2B-mCherry in NIH3T3 cells expressing GFP or GFP-Exportin 6.
 418 Images show representative coloured 3D surface reconstructions of daughter nuclei. Time
 419 stamp, h:min. **(d)** Quantification of nuclear volume during mitotic exit in NIH3T3 cells
 420 expressing either GFP or GFP-Exportin 6. Data shows mean \pm SEM from n=4 independent
 421 experiments and n=30 nuclei per condition. **(e)** Images of NIH3T3 cells stably expressing nAC-
 422 GFP (green) together with doxycycline-induced BFP-NLS or NLS-BFP-actin^{R62D} (gray, insets) at
 423 mitotic exit. Figures give proportion of cells showing nuclear F-actin (BFP-NLS, n=22; NLS-BFP-
 424 actin^{R62D}, n=18 mitotic events). Scale bar, 10 μm . **(f)** Visualization of nuclear expansion in
 425 NIH3T3 cells expressing doxycycline-induced BFP-NLS or NLS-BFP-actin^{R62D} similar to **c**. **(g)**
 426 Quantification of nuclear volume during mitotic exit in NIH3T3 cells expressing doxycycline-
 427 induced BFP-NLS or actin derivatives, as indicated. Data shows mean \pm SEM from n=3
 428 independent experiments and n=60 (BFP-NLS, NLS-BFP-actin^{R62D}), n=50 nuclei (Flag-NLS-actin^{wt},
 429 Flag-NLS-actin^{R62D}). **(h)** Representative AFM images show nuclear surface morphology of
 430 isolated NIH3T3 nuclei. Early G₁ nuclei were obtained from cells expressing Flag-NLS-actin^{wt} or

Flag-NLS-actin^{R62D}, whereas interphase nuclei were treated with or without TSA (1 μ M, 5 h) to induce chromatin decondensation. Scale bar, 1 μ m. **(i)** Quantification of surface roughness of early G₁ nuclei expressing Flag-NLS-actin^{wt} or Flag-NLS-actin^{R62D}. Data shows mean \pm SEM from n=2 independent experiments and n=9 nuclei per condition; P values were calculated by *t*-test. **(j)** Quantification of surface roughness of control or TSA-treated interphase nuclei. Data shows mean \pm SEM from n=2 independent experiments and n=7 nuclei per condition. P values were calculated by *t*-test.

Figure 4 | Nuclear actin dynamics promote chromatin decondensation at mitotic exit.

(a) NIH3T3 cells stably expressing a shuttling Actin Chromobody (sAC-GFP, green) together with H2B-mCherry. Asterisk indicates the nucleus shown magnified for additional time points to visualize nuclear F-actin within interchromatin spaces. See also Supplementary Video 3. Scale bars, 10 μ m; time stamp, min:s. **(b)** Maximum intensity projections of confocal z-stacks illustrate H2B-mCherry fluorescence densities (grey) during mitotic exit in NIH3T3 cells co-expressing BFP-NLS or NLS-BFP-actin^{R62D}. Scale bar, 10 μ m; time stamp, h:min. **(c-e)** Chromatin densities of NIH3T3 cells were assessed in G₀ or 90 min after anaphase (mitotic exit) and compared between **(c)** expression of BFP-NLS and NLS-BFP-actin^{R62D} (each n=60 nuclei), **(d)** expression of Flag-NLS actin^{wt} and Flag-NLS-actin^{R62D} (each n=50 nuclei) or **(e)** expression of GFP and GFP-Exportin 6 (each n=30 nuclei). In each panel data is shown as mean + SEM from n=3 independent experiments. P values were calculated by two-way ANOVA. **(f-i)** Fluorescence lifetime imaging microscopy (FLIM) of fluorescence resonance energy transfer (FRET) between GFP- and H2B-mCherry NIH3T3 cells at mitotic exit. **f** and **h** provide image examples for the

tested conditions. The colour code indicates fluorescence lifetime of GFP-H2B. Scale bar, 10 μm . **(g, i)** Quantifications of GFP-H2B fluorescence lifetime (Tau) in early G₁ NIH3T3 cells expressing either Flag-NLS-actin^{wt} or Flag-NLS-actin^{R62D} **(g)** or Flag-Exportin 6 **(i)**. Data is shown as boxplot of n \geq 20 **(g)** or n \geq 30 **(i)** cells per condition from n=3 independent experiments. P values were calculated by *t*-test. See also Supplementary Fig. 4a, b. **(j)** Representative electron microscopy images of cryo-preserved, synchronized early G₁ NIH3T3 cells induced to express BFP-NLS or NLS-BFP-actin^{R62D}. Areas indicated by a dashed rectangle are shown magnified. Scale bar, 2 μm . **(k, l)** Quantifications of condensed chromatin based on cryo-EM images. Chromatin condensation was compared between control conditions and expression of either NLS-BFP-actin^{R62D} (n \geq 28 nuclei) **(k)** or expression of GFP-Exportin 6 (n \geq 14 nuclei) **(l)**. In each panel data is shown as mean + SEM from n=2 independent experiments. P values were calculated by *t*-test.

Figure 5 | Inhibition of nuclear F-actin formation impairs transcription, proliferation and early embryonic development.

(a) 3D quantification of nuclear RNA Pol II pS5 fluorescence intensities in NIH3T3 cells expressing either GFP or GFP-Exportin 6 at indicated time points after mitotic shake off. See also Supplementary Fig. 5a. Data are shown as mean + SD from n=2 independent experiments and n \geq 10 nuclei per condition. P values were calculated by two-way ANOVA. **(b)** Proliferation of NIH3T3 cells expressing either GFP or GFP-Exportin 6 was measured using WST-1. Data were normalized to values of control cells at time 0 h and are shown as mean + SEM from n=3 independent experiments. P values were calculated by two-way ANOVA. **(c)** Experimental scheme for studying nuclear F-actin in fertilized mouse embryos. At 2 hpi (hours post

insemination) mRNA encoding for the indicated proteins was injected into fertilized embryos to either visualize or manipulate nuclear actin dynamics. **(d)** Image examples showing early embryo development at 8 hpi (pronuclei), 18 hpi and 24 hpi (2-cell). DIC, Differential interference contrast. Nuclear F-actin corresponding to dashed rectangles is shown magnified below (nAC-GFP, green). Scale bars, 20 μ m (overview) and 10 μ m (magnifications). **(e, f)** Image examples and quantitative analysis of nuclear volume in 2-cell embryos expressing either actin^{R62D}-HA-NLS or actin^{wt}-HA-NLS **(e)** or mCherry-Exportin 6 **(f)**. Scale bar, 20 μ m. Quantitative data is shown as mean + SD from n=3 independent experiments and n \geq 30 nuclei per condition. P values were calculated by *t*-test. **(g)** Preimplantation development of embryos after a single injection of mCherry-Exportin 6 mRNA. Note the significant developmental delay at 48 hpi, 60 hpi, 72 hpi and 96 hpi compared to injection of mRNA encoding myc-tagged GFP. Data is shown as mean + SEM from n=3 independent experiments. P values were calculated by chi-squared test. See also Supplementary Fig. 5b.

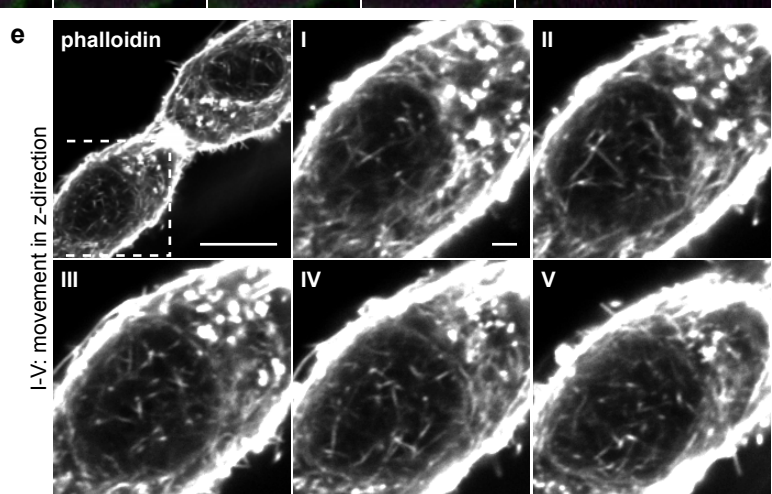
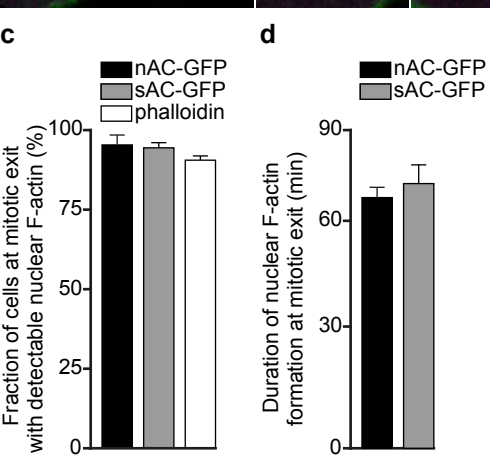
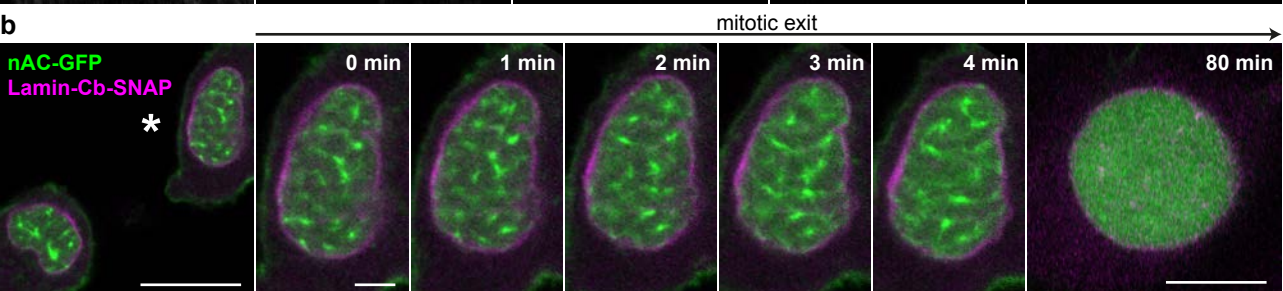
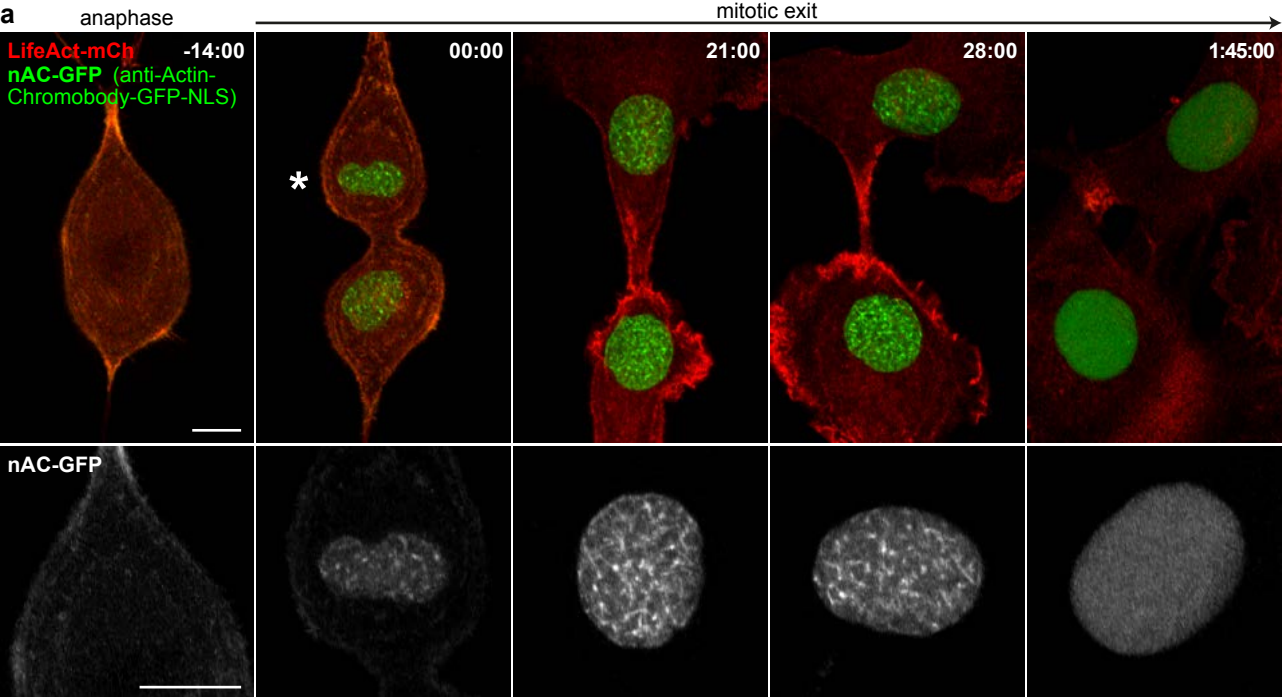
Figure 6 | Nuclear Cofilin-1 regulates nuclear F-actin during mitotic exit.

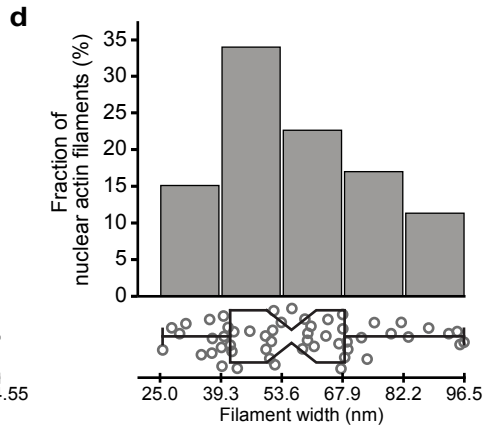
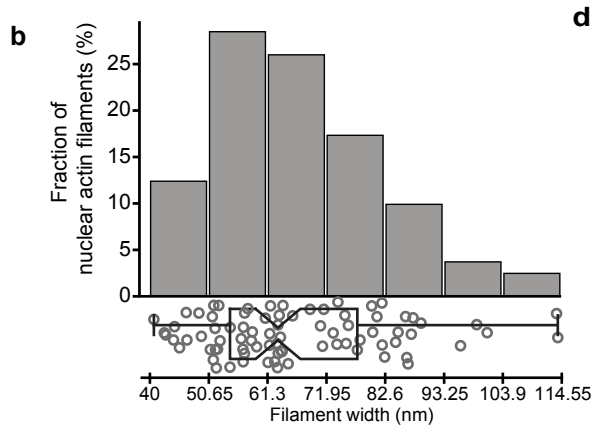
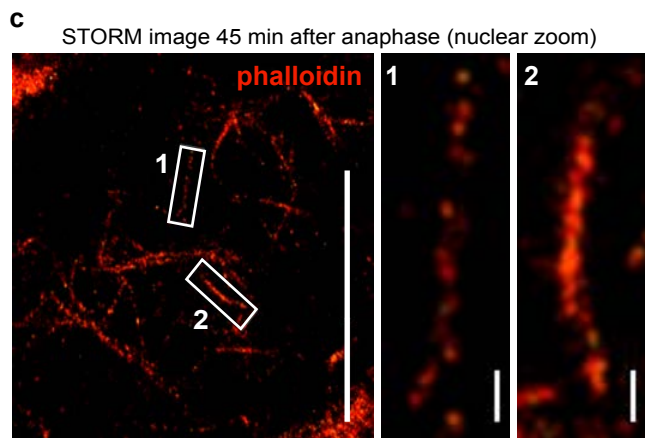
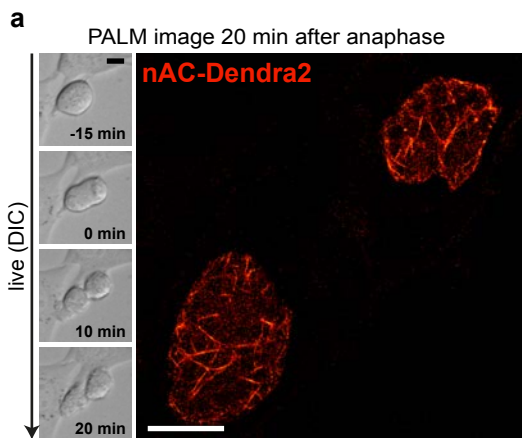
(a) Cartoon illustrating nuclear F-actin pulldown at mitotic exit. **(b)** Immunoblot detecting α -Tubulin (cytoplasm) and Histone 3 (H3, nucleus) confirms successful subcellular fractionation. **(c)** Immunoblot detecting β -actin and Cofilin-1 validates efficient pulldown of nuclear F-actin and co-precipitation of Cofilin-1. **(d)** Table listing nuclear F-actin-binding proteins as identified by mass spectrometry (cov., coverage). **(e)** 3D quantitative immunofluorescence analysis of nuclear p-Cofilin at indicated times after mitotic shake-off. Data are shown as mean + SD from n=30 mitotic events for each time point. See also Supplementary Fig. 6a, b. **(f)** Time-lapse

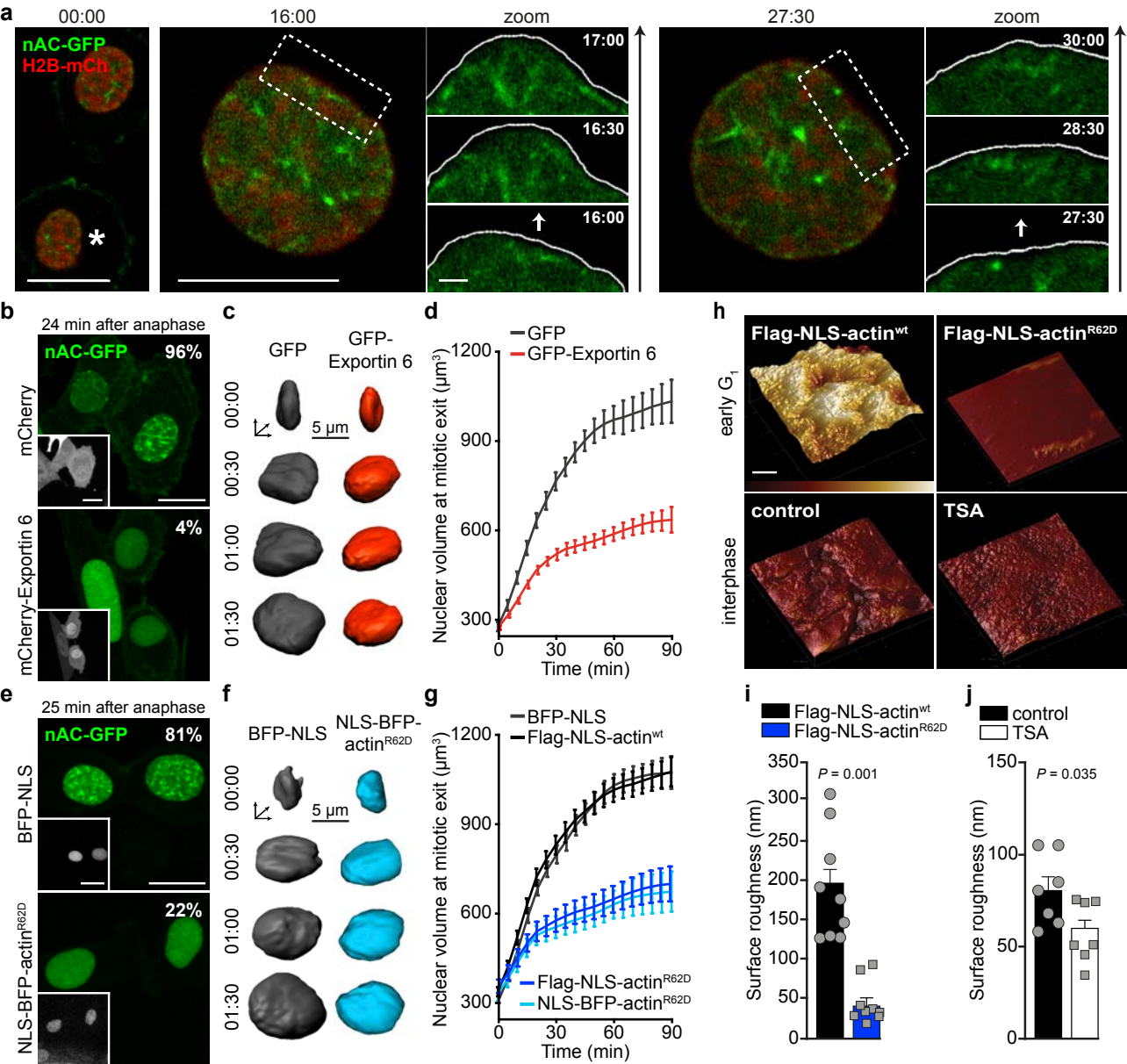
imaging shows nAC-GFP expressing NIH3T3 cells transfected with si-control or si-Cofilin at indicated times after anaphase. See also Supplementary Video 5. Scale bar, 5 μ m; time stamp, h:min. **(g)** Quantifications of stabilized nuclear F-actin (present for ≥ 2 h after anaphase) in NIH3T3 cells treated with indicated siRNAs. Data are shown as mean + SD from n=3 independent experiments and n=49 (si-control), n=58 (si-Cofilin), n=59 (si-Cofilin (3'-UTR)) mitotic events. Immunoblot confirms efficient silencing of Cofilin-1. **(h)** Confocal images of fixed NIH3T3 cells stably expressing WT- or NES-mCherry-Cofilin (red). Scale bar, 10 μ m. **(i)** Immunoblot of NIH3T3 cells stably expressing WT- or NES-mCherry-Cofilin confirms siRNA (si-Cofilin (3' UTR))-resistant expression of mCherry-Cofilin derivatives. **(j)** Quantifications of stabilized nuclear F-actin (present for ≥ 2.5 h after anaphase) in NIH3T3 cells treated with si-Cofilin (3'-UTR) in the presence of either WT- or NES-mCherry-Cofilin. Data are shown as mean + SD from n=3 independent experiments and n=30 (WT), n=38 (NES) mitotic events. **(k)** Quantifications of nuclear volume during mitotic exit in NIH3T3 cells treated with si-control or si-Cofilin (3'-UTR) in the presence of either WT- or NES-mCherry-Cofilin. Data are shown as mean + SD from n=3 independent experiments and n=50 nuclei per condition. **(l)** NIH3T3 cells expressing WT- or NES-mCherry-Cofilin were treated with si-control or si-Cofilin (3'-UTR) and chromatin densities were assessed 90 min after anaphase. Data are shown as mean + SEM from n=3 independent experiments and n=50 nuclei per condition. Unprocessed original scans of blots are shown in Supplementary Fig. 7.

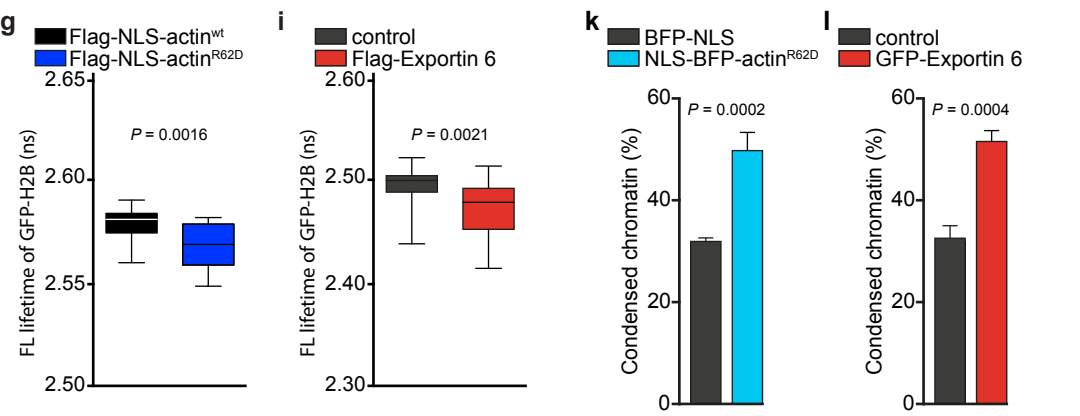
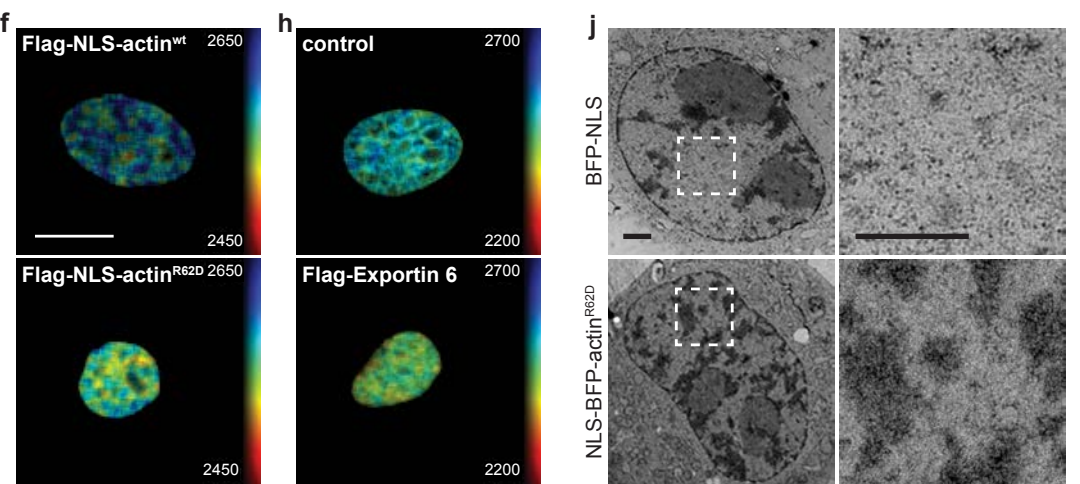
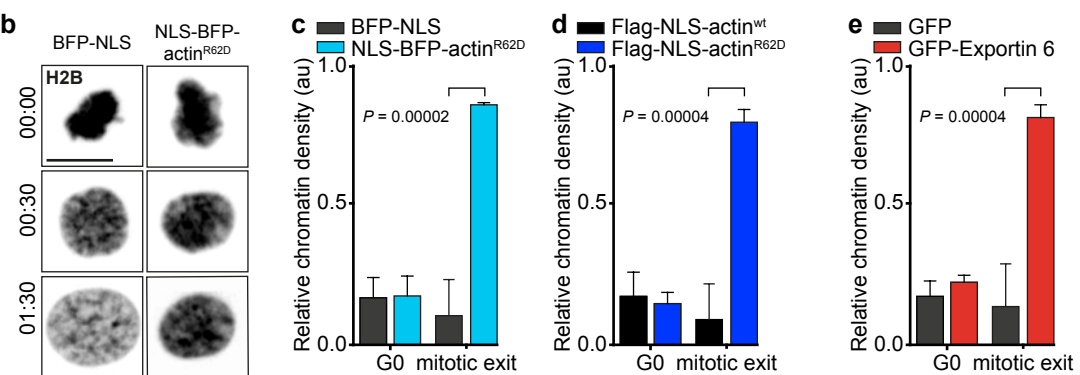
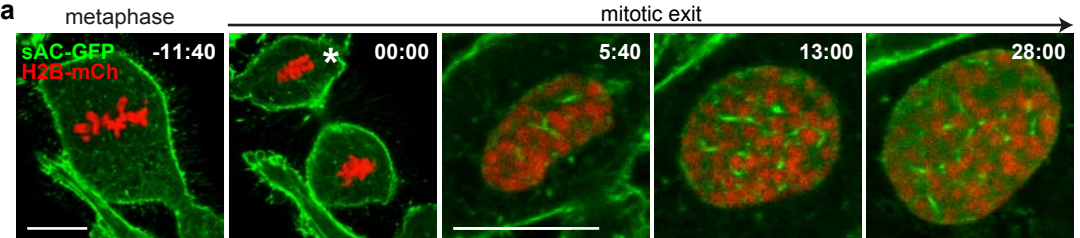
Figure 7 | Nuclear Cofilin-1 affects chromatin organization and its optogenetic control reveals critical functions in nuclear F-actin disassembly at mitotic exit.

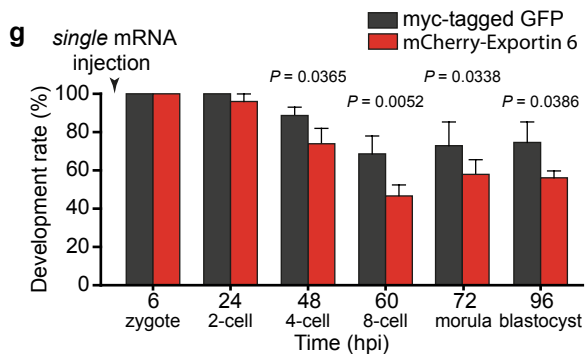
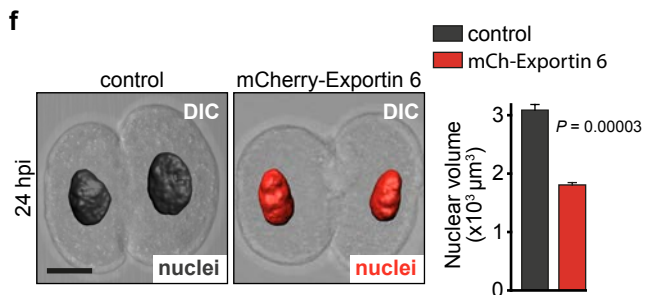
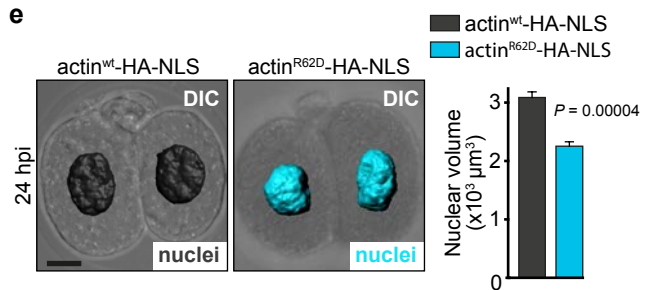
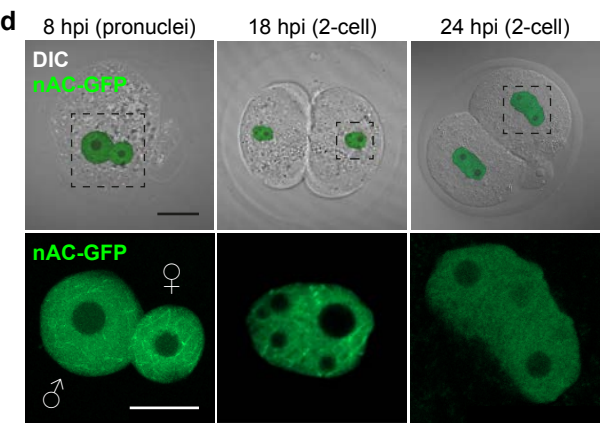
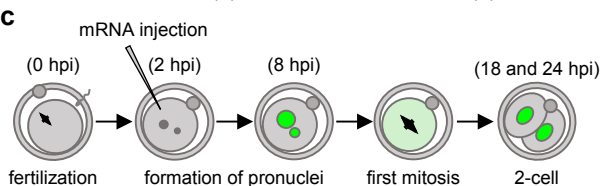
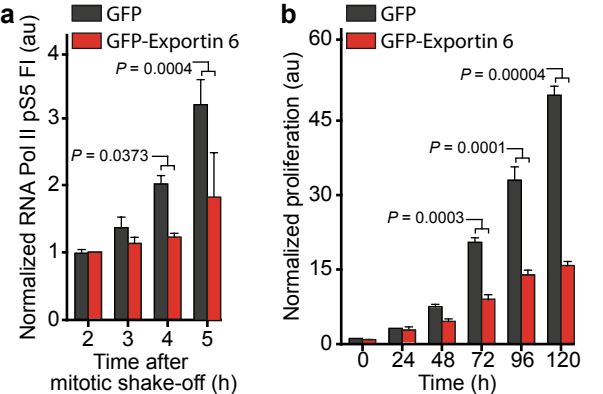
(a) Representative electron microscopy images of cryo-preserved NIH3T3 cells at mitotic exit in the absence or presence of NLS-mCherry-Cofilin. Scale bar, 2 μ m. **(b)** Quantifications of condensed chromatin based on cyro-EM images. Data are shown as mean + SEM from n=2 independent experiments and n \geq 15 nuclei per condition. P values were calculated by t-test. **(c)** Cartoon illustrating design and photo-convertibility of opto-Cofilin. Blue light induces exposure of a photocaged NES (nuclear export sequence), thereby promoting its nuclear export. **(d)** Time-lapse imaging of NIH3T3 cells demonstrates light-regulated control of opto-Cofilin subcellular localization. NIH3T3 cells stably expressing opto-Cofilin (grey) were imaged at 10 second intervals either with (+ light) or without (- light) additional irradiation by blue laser light (488 nm). See also Supplementary Video 6. Scale bar, 10 μ m. **(e)** NIH3T3 cells stably expressing nAC-SNAP (labelled by SiR-647, grey) and opto-Cofilin (red) were treated with si-Cofilin (3'-UTR) and imaged during and after mitosis. To promote nuclear export of opto-Cofilin, cells were exposed to blue laser light (488 nm) at 2.5 min intervals (+ light). Note the excessive formation of stabilized nuclear F-actin in the absence of nuclear Cofilin as well as the onset of filament disassembly upon controlled nuclear re-import of opto-Cofilin (- light). See also Supplementary Video 8. Scale bar, 10 μ m. **(f)** Nuclear fluorescence intensities of opto-Cofilin (red line) and relative nuclear area (black line) were quantified before and during light-regulated re-import of opto-Cofilin. Nuclear re-import of opto-Cofilin is accompanied by nuclear shape changes and overall nuclear expansion. Data are shown as mean from n=5 nuclei.

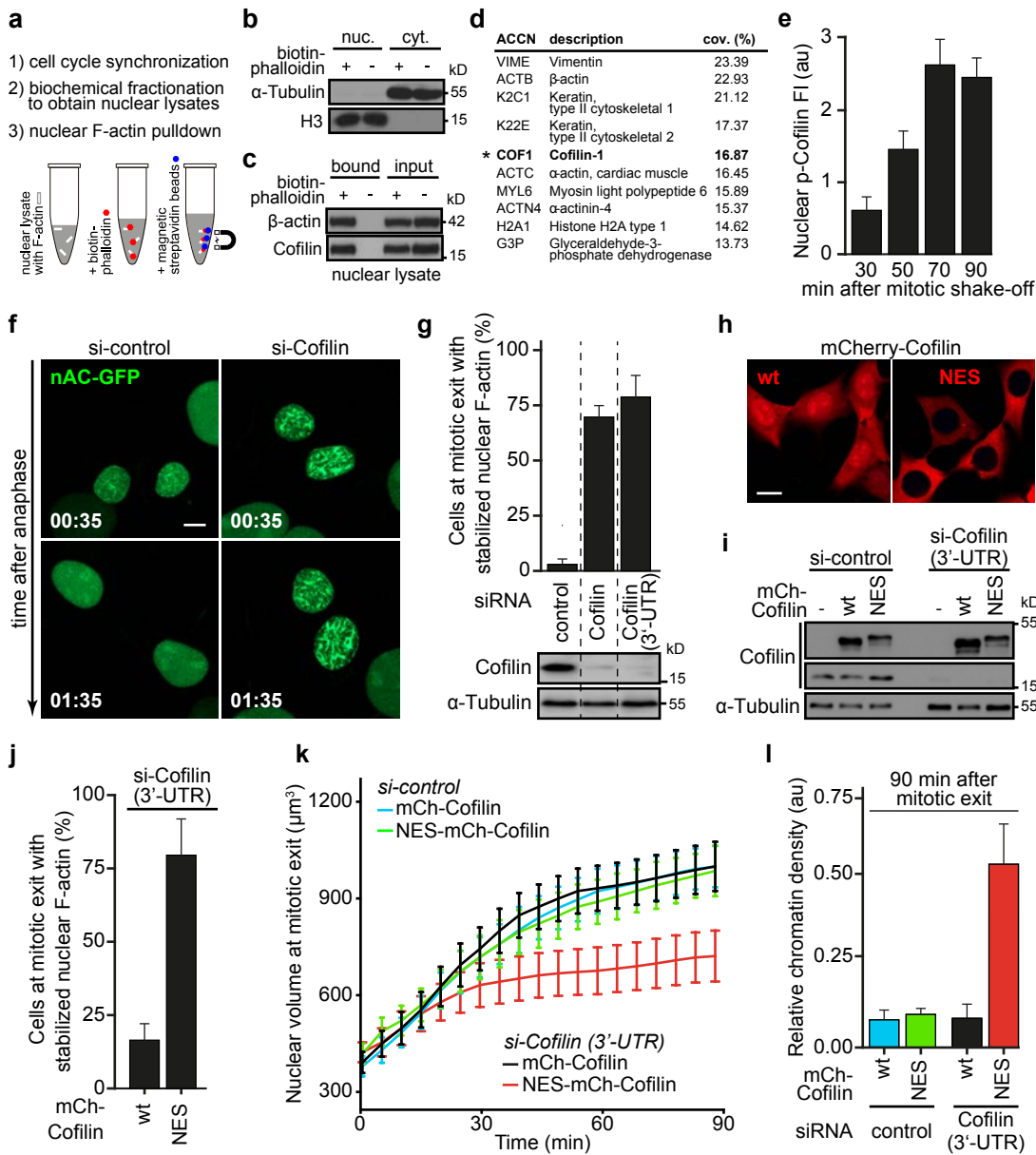


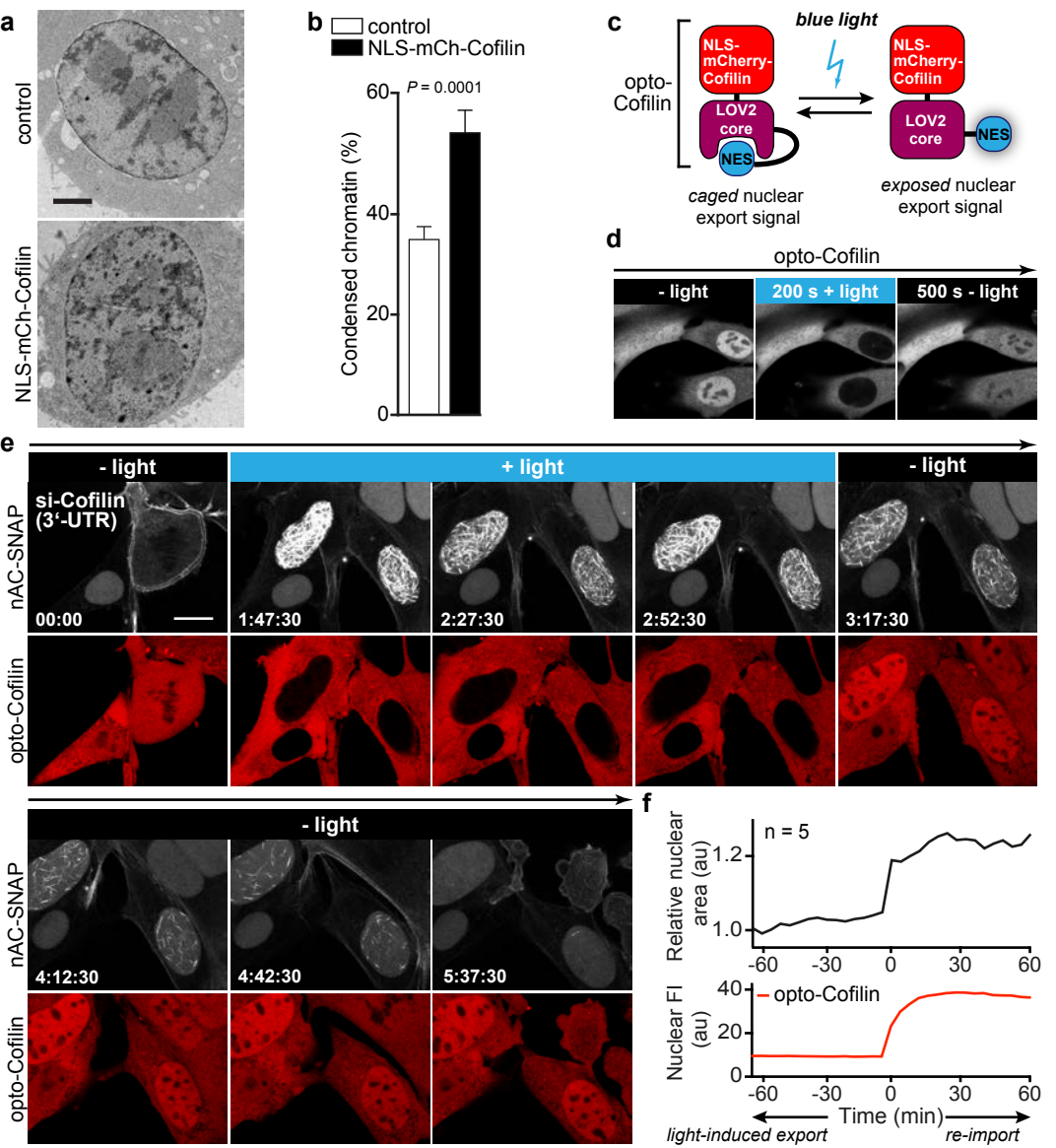












1 **Methods**

2 **Antibodies and reagents**

3 Cell culture and transfection reagents (Lipofectamine 2000 and RNAiMax) were obtained
4 from Invitrogen.

5 Rhodamine-phalloidin, biotin-phalloidin, phalloidin-AF647 and phalloidin-Atto 488
6 were purchased from Life Technologies. SNAP-Cell 647-SiR was obtained from NEB. SiR-DNA
7 was from Spirochrome. CK-666, Cytochalasin D, DMSO, doxycycline, Latrunculin B, and
8 nocodazole were from Sigma-Aldrich and used at indicated concentrations. Flavopiridol was
9 obtained from Santa Cruz and used at a final concentration of 1 μ M.

10 Information on antibodies used is listed in Table S3.

11

12 **Plasmids and constructs**

13 The generation of nuclear Actin-Chromobody-GFP (nAC-GFP, anti-actin-Chromobody-GFP-
14 NLS) and sAC was described previously ⁸. The nuclear Actin-Chromobody is a genetically
15 encoded, NLS-tagged nanobody against the actin protein. Due to its NLS-fusion, the nuclear
16 Actin-Chromobody is enriched in the nuclei of interphasic cells. Nuclear envelope
17 breakdown in prophase is accompanied by a temporary loss of its specific nuclear
18 localization, which reestablishes during mitotic exit and daughter nuclei assembly.

19 nAC-Dendra2, nAC-mCherry and nAC-SNAP were generated by replacing the GFP of
20 pWPXL-nAC-GFP by either pDendra2 (Clontech Laboratories, Inc.), mCherry or the SNAP-tag
21 (New England Biolabs).

22 To obtain a Lamin-nanobody fused to the SNAP-tag, the tagRFP2 of pLC-TagRFP2
23 (ChromoTek) was replaced by a cDNA encoding the SNAP-tag. For stable expression of

24 Lamin-nanobody-SNAP, the corresponding cDNA was subcloned into pWPXL using the
25 MluI/SpeI restriction sites.

26 For stable expression of H2B-mCherry, the GFP of pWPXL was replaced by mCherry,
27 before insertion of a cDNA encoding human H2B via the BamHI/MluI restriction sites.

28 For generation of tagRFP-KASH, the cDNA of murine Nesprin-1 α was amplified as described
29 ¹⁷. KASH was expressed from the EFpLink plasmid carrying a N-terminal tagRFP.

30 The cDNA of human Exportin 6 was obtained by reverse transcription of total mRNA
31 obtained from HeLa cells. Exportin 6 was expressed from the EFpLink plasmid carrying a N-
32 terminal Flag-, Flag-GFP- or mCherry-tag.

33 To obtain mCherry-Cofilin plasmids for lentiviral transductions, the cDNA of mouse
34 Cofilin-1 (kindly provided by M. Rust, University of Marburg) was N-terminally fused to
35 mCherry and cloned into pWPXL via MluI/SpeI. For NES-mCherry-Cofilin the NES (nuclear
36 export signal) of HIV1-Rev (LPPLERLTL) was fused to the N-terminus of mCherry. Opto-
37 Cofilin was generated by addition of the cMyc^{P1A} NLS (AAAKRVKLD) to the N-terminus of
38 mCherry-Cofilin and a C-terminal fusion to the LEXY module ²⁶. Opto-Cofilin was inserted
39 into pWPXL via MluI/SpeI to allow for production of lentiviral particles.

40 To obtain NLS-BFP-actin^{R62D}, the SV40 large T antigen NLS (PPKKKRKV) was N-
41 terminally fused to tagBFP2 (separated by one linking glycine), which was further fused to
42 the N-terminus of actin^{R62D} ¹⁸, separated by a SGLRSRA linker. For BFP-NLS, the cDNA
43 encoding tagBFP2 was C-terminally fused to the SV40 large T antigen NLS, separated by a
44 GDPPVAT linker. To obtain Flag-NLS-actin-T2A-SNAP derivatives, a cDNA encoding human β -
45 actin (either wild-type or containing the point mutation R62D) was N-terminally fused to a
46 Flag-tag and the SV40 large T antigen NLS (separated by a BamHI restriction site) and C-
47 terminally linked to a SNAP-tag by a self-cleaving T2A peptide (GSGEGRGSLTCTGDVEENPGP).

48 To allow for stable doxycycline-inducible expression of NLS-BFP-actin^{R62D}, BFP-NLS or Flag-
49 NLS-actin-T2A derivatives, the corresponding cDNAs were inserted into the pInducer20
50 plasmid²⁸ by homologous recombination using the Gateway technology (Invitrogen).
51 LifeAct-mCherry lentiviral particles were a gift from O. Fackler (University of Heidelberg).

52

53 **Cell culture, viral transductions, transfections, and treatments**

54 NIH3T3, HT1080, RPE-1 cells and all their derivatives were grown in DMEM supplemented
55 with 10% FCS (fetal calf serum), 100 U/ml penicillin and 100 mg/ml streptomycin at 37 °C in
56 a 5% CO₂ atmosphere. MCF10A cells and derivatives were cultured as described previously
57²⁹.

58 Lentiviral transductions were performed as previously described⁸.

59 Transient transfections of Exportin 6 or tagRFP-KASH were carried out using
60 Lipofectamine LTX&PLUS Reagent according to the manufacturer's instructions.
61 Transfection of siRNAs and sequence for si-Emerin and si-Lamin A/C have been described
62 previously⁸. In addition, the following siRNAs were obtained from Qiagen:
63 CCGCTGCACCCTGGCAGAGAA (si-Cofilin), TGCCAACTTCTAACCACAATA (si-Cofilin (3'-UTR)),
64 and TTGGACTATCTGACAAGTAAA (si-Exportin 6). Sequences of siRNAs used to obtain data
65 presented in Table S1 are shown within the table.

66 SNAP-Cell 647-SiR and SiR-DNA were used according to the manufacturer's
67 instructions.

68 Induction of BFP-NLS, NLS-BFP-actin^{R62D} or Flag-NLS-actin-T2A-SNAP derivatives was
69 achieved by addition of 0.5 µg/ml doxycycline for 16 hours to the cell culture medium.
70 NIH3T3 cells were arrested in G₀ phase by serum starvation (growth medium without FCS)
71 for at least 16 hours.

72

73 **Immunofluorescence and phalloidin staining**

74 For immunofluorescence stainings, cells were grown on cover slips, before fixation using 4%
75 formaldehyde (15 min at RT). Immunolabeling was performed as described previously²⁹.

76 For phalloidin staining in Fig. 1e, cells were fixed using glutaraldehyde according to⁷.
77 Phalloidin staining for the quantification shown in Fig. 1c and for super-resolution
78 microscopy (Fig. 2c,d and Supplementary Fig. 2c) was performed on formaldehyde fixed
79 samples (4% for 15 min at RT). After washing with PBS, samples were incubated with
80 phalloidin-AF647 or phalloidin-Atto 488 at 4°C for 96 hours. For super-resolution
81 microscopy, samples were post-fixed using 1% formaldehyde for 10 min at RT.

82

83 **Image acquisition and live cell imaging**

84 All confocal image acquisitions were performed on a LSM 700 or LSM 800 confocal laser
85 scanning microscope (Zeiss) equipped with a 63X/1.4 NA oil objective. For live cell imaging,
86 cells were cultured in μ -slides (ibidi) at 37 °C in a 5% CO₂ atmosphere using a humidified
87 incubation chamber (Pecon).

88 Experiments with opto-Cofilin were carried out using the LSM 800 microscope. To
89 induce nuclear export of opto-Cofilin the excitation of mCherry (555 nm) was combined
90 with 488 nm irradiation at 1% laser power and a pixel dwell of 2.06 μ sec.

91

92 **Image processing and quantification of nuclear volumes, chromatin densities and 3D** 93 **fluorescence intensities**

94 Image processing was performed with IMARIS (Bitplane), FIJI (NIH) and Photoshop CS6
95 (Adobe).

For quantification of nuclear volume in living NIH3T3 cells, images were acquired every 2.5 or 5 min over an interval of 90 min. Complete z-stacks of the signal obtained by either H2B-mCherry (Fig. 3c, d, f, g) or SiR-DNA (Fig. 6k; Supp. Fig. 3a, b) were analyzed with IMARIS. 3D surfaces based on the nuclear-specific signal were generated, and their respective volumes were measured over time. Chromatin density was calculated by dividing the sum of H2B or SiR-DNA fluorescence intensities by total nuclear volume.

For measuring nuclear fluorescence intensities in 3D, a nuclear staining (i.e. DAPI) was used to threshold a nuclear region of interest (with FIJI for 2D data, and IMARIS for 3D data), from which the respective pixel intensities were calculated.

PALM and measurement of nuclear actin filament width

For super-resolution imaging of nAC-Dendra2, NIH3T3 cells were washed and fixed in 3.7 % formaldehyde (FA), 20 min at room temperature. For all super-resolution imaging, a 1:5000 dilution of fluorescent beads (TetraSpeck™ Microspheres, 0.1 μm, Life Sciences T7279 or FluoSpheres 715/755, Life Sciences F8799) was sonicated to break up clumps of beads. ~5 μL of the beads were added to the sample and allowed to settle and adhere for 15 minutes, to serve as fiducial markers for drift correction. Super-resolution imaging was performed as described in detail elsewhere³⁰. Briefly, a customized and automated Nikon Eclipse Ti microscope was equipped with 405 nm, 488 nm, 561 and 640 nm lasers (all OBIS, Coherent Inc.). Laser intensities were controlled by an acousto-optical tunable filter (Acal BFi Germany GmbH) to illuminate the sample using the quad color dichroic zt405/488/561/640rpc. The illumination could be switched from epifluorescence to total internal reflection fluorescence (TIRF) mode by a motorized TIRF mirror (Thorlabs, Germany). Fluorescence signals were collected by a quad line laser rejection filter ZET405/488/561/640 and the bandpass filters

ET 525/50, ET 610/75 or HC 689/23 dependent on the imaging channel (all filters AHF, Germany). The detection of the single fluorophore emissions was performed using an electron multiplying charged coupled device (emCCD iXon 888, Andor).

For PALM imaging of Dendra2, the sample was irradiated by about 1-2 kW/cm² (561 nm) and < 0.5 kW/cm² (405 nm) and imaged at an acquisition frame rate of 60 ms. Cells were imaged until all Dendra2 fluorophores were read-out. For STORM imaging of Phalloidin-Alexa 647, the sample was imaged in 100 mM Methyl diethanolamine (MEA) with a glucose oxidase oxygen scavenger system³¹ illuminated with about 2-4 kW/cm² (640 nm) and recorded at an acquisition frame rate of 70 ms.

Super-resolution movies were analyzed by the RapidSTORM software³² and post-processed by customized scripts written in Python programming language (Python Software Foundation, <https://www.python.org/>) to correct for sample drift during the image acquisition. From the post-processed data, the experimental Nearest Neighbor Approach (NeNA) resolution³³ was calculated and super-resolution images were reconstructed according to their individual resolution.

Filament widths were analyzed by a self-written, customized script for the FIJI software³⁴. Briefly, the filaments were selected by a segmented line profile covering the filament width and length. To minimize the selection and pixelation error, selections were shifted by 0.5 pixels (5 nm) in all directions to obtain five measures in total for each filament. These selected ROIs were straightened to remove the individual curvatures of the filaments and projected along their long axis. The obtained profiles were fitted by a Gaussian which yields the filament widths by its FWHM-value. The optimal histogram bin size was calculated using the Freedman – Diaconis rule³⁵.

Fluorescent lifetime imaging microscopy (FLIM) of fluorescence resonance energy transfer (FRET)

For FLIM/FRET, NIH3T3 cells were transduced with lentiviruses encoding PGK-H2B-mCherry and PGK-GFP-H2B, and sorted for homogeneous expression. For experiments involving expression of Exportin 6, cells were seeded and synchronized by a single thymidine block, 24 hours after transfection of Flag-Exportin 6. After this, cells were washed 3x in PBS and cultured in normal media for 10 hrs. Cells were then fixed (2% PFA in PBS, 10 min), permeabilised (0.1% Triton in PBS, 10 min), and blocked (2% BSA in PBS, 30 min), before incubation with anti-Flag antibodies for 2 hours. Cells were washed 3x in PBS, and incubated with secondary Alexa Fluor 405-conjugated antibodies for 45 minutes. Cells were then washed again in PBS. Post-mitotic, Flag-Exportin 6-overexpressing cells were identified using 405 nm excitation.

In the case of Flag-WT/R62D mutant actin, transfected cells expressing Flag-WT/R62D-NLS-actin were sorted through SNAP-mediated tagging. Following this, cells were transduced to express GFP-H2B and mCherry-H2B, and sorted by FACS. For these experiments, cells were synchronised at G1/S transition using thymidine (2 mM for 20 hours). Cells were then washed three times in PBS, and returned to normal media containing 500 ng/ml Doxycycline to induce expression of Flag-actin derivatives. After 4 hours, 1 μ M of CDK1i (RO-3306, Sigma) was added to the medium for a subsequent period of 4 hours. Cells were washed three times in PBS, and then returned to normal media containing 500 ng/ml Doxycycline. 30 min later, mitotic cells were isolated by mitotic shake off, centrifuged at 1500 x g for 5 min and plated onto PLL-coated 35 mm dishes. After one hour cells were pre-extracted with CSK buffer for 5 minutes, washed three times in PBS and fixed with 2% PFA.

Lifetime measurements were taken on a Leica TCS SP8 system, using a white light laser with a repetition rate of 20 MHz and an excitation wavelength of 488 nm. GFP-H2B emission was detected over an emission range of 495 – 530 nm. Data was fitted using FLIMfit software³⁶. Temporal binning of the fluorescence decays was performed prior to fitting, resulting in 256 time bins per decay. Tail-fitting of the fluorescence images was performed pixel-wise with a single exponential model on all pixels above an intensity threshold of 175 photons, allowing spatial variations in fluorescence lifetime to be visualized.

Nuclear F-actin pulldown at mitotic exit

For nuclear F-actin pulldowns, RPE-1 cells were mitotically blocked by nocodazole (100 nM for 24 hours), before washout with growth medium. 4 hours after washout, cells were lysed and subjected to subcellular fractionation as described previously⁸. Purity of subcellular fractionations was controlled by immunoblotting for α -Tubulin and histone H3. The obtained nuclear lysates were incubated with 5 μ g biotin-phalloidin at 4 °C and constant rotation for 2 hours. Then, pre-washed magnetic streptavidin dynabeads (Thermo Fisher) were added, following incubation at 4 °C and constant rotation for 2 hours. After washing, the magnetic beads were collected and boiled in 2x Laemmli buffer for 10 min. The supernatant containing lysed nuclear F-actin and associated proteins was used for further analyses.

Mass spectrometry-based protein identification

192

193 Samples were loaded on an SDS gel and immediately after they had entered the separation
194 gel, electrophoresis was stopped and the protein bands were excised and subjected to in-gel
195 digest using trypsin ³⁷.

196 For mass spectrometric analysis an Orbitrap Velos Pro mass spectrometer
197 (ThermoScientific) was used which was connected online with an Ultimate nanoRSLC-HPLC
198 system (Dionex), equipped with a nano C18 RP column. 10 µL of the tryptic digest were
199 usually injected onto a C18 pre-concentration column and automated trapping and
200 desalting of the sample was performed at a flowrate of 6 µL/min using water/0.05% formic
201 acid as solvent.

202 Tryptic peptides were separated with water/0.045% formic acid (solvent A) and 80%
203 acetonitrile/0.05% formic acid (solvent B) at a flow rate of 300 nL/min: holding 4% B for five
204 minutes, followed by a linear gradient to 45% B within 30 minutes and linear increase to
205 95% solvent B for 5 minutes. The column was connected to a stainless steel nanoemitter
206 (Proxeon, Denmark) and the eluent sprayed directly towards the heated capillary of the
207 mass spectrometer using a potential of 2300 V. A survey scan with a resolution of 60000
208 within the Orbitrap mass analyzer was combined with at least three data-dependent MS/MS
209 scans with dynamic exclusion for 30 s either using CID with the linear ion-trap or using HCD
210 and Orbitrap detection at a resolution of 7500.

211 Data analysis was performed using Proteome Discoverer (v4.0; ThermoScientific)
212 with SEQUEST and MASCOT (v2.4; Matrix science) search engines using either SwissProt or
213 NCBI databases.

214

215 **Mitotic shake-off**

For indicated immunoblot analyses, immunofluorescence staining and MNase assays, cells were seeded at 40% confluency and allowed to adhere for 8 hours. Cells were then serum starved for 24 hours, followed by addition of growth media, containing 0.33 µg/ml doxycycline for experiments involving dox-inducible protein expression. After 16 hours, nocodazole (100 nM) was added for 3 additional hours. Mitotic cells were collected by mitotic shake-off and washed three times in growth media. These mitotic cells were then reseeded and further processed for subsequent analyses.

Micrococcal nuclease (MNase) digestion assay

One million cells were harvested, and washed once with 1 ml of 1x RSB buffer (10 mM Tris, pH 7.6, 15 mM NaCl, and 1.5 mM MgCl₂). After centrifugation (3,000 x g), the cell pellet was resuspended in 1 ml of 1x RSB buffer with 1% Triton-X 100 and homogenized. Nuclei were collected by centrifugation (13,000 x g) and washed twice with 1 ml of buffer A (15 mM Tris, pH 7.5, 15 mM NaCl, 60 mM KCl, 0.34 M sucrose, and 0.1% β-mercaptoethanol, EDTA-free protease inhibitor cocktail). Nuclei were resuspended in 500 µl MNase reaction buffer (from NEB, 50 mM Tris-HCl, 5 mM CaCl₂ pH 7.9) and aliquoted into 100 µl aliquots. MNase digestion was performed in 100 µl reactions by addition of 50 Kunitz units of MNase (NEB) at 37 °C for 5 minutes. Reactions were terminated by adding 25 mM EDTA. DNA was purified using a PCR purification kit and 1000 ng of DNA was analyzed on a 1.5% agarose gel.

Animals

ICR mice were obtained from Kiwa Experimental Animals (Wakayama, Japan). This study conformed to the Guide for the Care and Use of Laboratory Animals. All animal experiments

were approved and performed under the guidelines of the Animal Research Committee of Kindai University.

***In vitro* fertilization of mouse oocytes and mRNA injection**

Female ICR mice (or ICR x ICR), aged 8-13 weeks, were superovulated with pregnant mare serum gonadotropin (PMSG; Novartis Animal Health, Japan), followed 48 hours later with human chorionic gonadotropin (hCG; ASKA Pharmaceutical). Cumulus–oocyte complexes were collected from the oviducts in HTF medium. The sperm suspension was added to the oocyte cultures, and morphologically normal fertilized oocytes were collected 1-1.5 hours after insemination at 37°C under 5% CO₂ in air. Fertilized oocytes were transferred to HEPES-CZB medium. mRNAs for nAC-GFP, actin^{R62D}-HA-NLS, actin^{wt}-HA-NLS, mCherry-Exportin 6, myc-GFP were injected using a piezo manipulator (Prime Tech). mRNAs were prepared from pCS2³⁸ or pcDNA3.1 vectors³⁹. In the case of pCS2 vectors, mRNAs produced from the SP6 promoter were subjected to the addition of polyA tails while pcDNA3.1 vectors were transcribed from the T7 promoter. Since the translation efficiency is different between mRNAs produced from pCS2 vectors and those from pcDNA3.1 vectors, different concentrations of mRNA were injected; nAC-GFP (150 ng/μl), HA-NLS-actin^{R62D} (650 ng/μl), HA-NLS-actin^{wt} (650 ng/μl), mCherry-Exportin 6 (1,000 ng/μl) and myc-GFP (1,000 ng/μl), histone H2B-mCherry (5 ng/μl). After mRNA injections, the fertilized embryos were cultured in KSOM medium at 37°C in a 5% CO₂ atmosphere. mCherry-Exportin 6 mRNA or control myc-tagged GFP mRNA was injected into oocytes denuded by 0.1% hyaluronidase before subsequent *in vitro* fertilization.

Confocal microscopy of oocytes

mRNA-injected embryos were fixed in 4% formaldehyde for 10 min and washed three times in PBS containing 0.01% PVA. Then, fixed embryos were incubated in PBS-BSA with 0.2% Triton-X for 60 min, followed by three time washes with PBS-BSA. Washed embryos were stained with 5 µg/ml DAPI for 15 min, followed by three times washing using PBS-BSA. Embryos were mounted and observed under a confocal microscope (LSM800, ZEISS). Images were analyzed using the ZEN software (ZEISS).

Electron microscopy (EM)

For EM-based analysis of chromatin compaction at mitotic exit, NIH3T3 cells (either stably expressing BFP-NLS, NLS-BFP-actin^{R62D} or transfected with GFP-Exportin 6 or NLS-mCherry-Cofilin) were synchronised at G1/S transition using thymidine (2 mM for 20 h). Expression of GFP-Exportin 6 and NLS-mCherry-Cofilin was ensured by FACS-based cell sorting prior to sample preparation. Cells were washed three times in PBS, and then returned to normal media containing 500 ng/ml Doxycycline. After 4 hours, analysis of flow cytometry data, using the Watson (Pragmatic) model, determined that 40% of cells had completed S phase. This time point was therefore chosen to add 1 µM of CDK1i (RO-3306, Sigma), for a period of 4 hours. Cells were washed three times in PBS, and then returned to normal media containing 500 ng/ml Doxycycline. 30 min later, mitotic cells were isolated by mitotic shake off. Cells were centrifuged at 1500 x g for 5 min, and plated onto PLL-coated 35 mm dishes.

After 1 hour, cells were trypsinised and centrifuged at 1500 x g for 5 min. Pellets were re-suspended in complete media containing 10% BSA, and centrifuged at 1500 x g for 5 min. 1 µl of this cell pellet was then put into a 0.1 mm gold membrane carrier and high pressure frozen (Leica EM PACT2). Samples were then freeze-substituted in a freeze-substitution acetone mix, containing 0.1% uranyl acetate and 1% osmium tetroxide. During

this procedure, samples were first held at -90°C, then brought to 0°C, over a period of 18 hrs. These samples were then embedded in EPON, and baked at 60°C for 48 hrs. 70 nm sections were cut using an ultratome, which were stained with uranyl acetate and lead citrate and images were taken at 2900x magnification on a FEI Tecnai 12 TEM, operated at 120kV.

For analysis, nuclei and nucleoli were manually segmented in 2D slice images across the cell using the freehand selection tool of ImageJ/Fiji to generate a binary mask of the nucleoplasm. Condensed chromatin was then semi-automatically segmented across the nucleoplasmic region using the WEKA Trainable Segmentation plugin for ImageJ/Fiji ⁴⁰. Classification was based on the Gaussian blur, Sobel filter, Hessian, Difference of Gaussians and membrane projections metrics using the built-in fast random forest algorithm. Due to the variability in chromatin staining, it was necessary to train a new classification model for each image. Condensed chromatin distribution was subsequently analysed in the segmented images using a custom ImageJ/Fiji macro, which measured the total condensed chromatin area and perimeter, as well as the area fraction of condensed chromatin, as a proportion of the total nucleoplasmic area.

Atomic force microscopy

To obtain early G1 or interphase NIH3T3 nuclei, cells were collected 60 min or 7 hours after mitotic shake-off, respectively. Nuclei were isolated as described previously ⁴¹. Atomic force microscopy measurements were conducted in aqueous solution utilizing a Multi-mode VIII microscope with Nanoscope V controller and a PeakForce feedback control mechanism with an enclosed liquid cell. Isolated live nuclei were bound to a poly-L-lysine coated glass coverslip and remained hydrated in this buffer (20 mM HEPES at pH 7.8, 25 mM KCl, 5 mM

MgCl₂, 0.25 M sucrose and 1 mM ATP) to increase the longevity of the nuclei for investigation. The surface morphologies of nuclei were observed to remain unchanged under these conditions, allowing multiple nuclei to be tested in each sample and an average surface roughness and associated error to be calculated for each nucleus type. Using SCANASYST-FLUID cantilevers [Bruker, CA, USA] of nominal spring constant 0.7 N/m and nominal tip radius 2 nm, the force applied to plane of the sample by the AFM tip was kept below 1 nN, thus imaging stability was maintained whilst avoiding tip-induced deformation of the sample. Images were collected at a scan rate of 0.404 Hz with at 500 x 500 pixels giving a digital resolution of ~10 nm/pixel. Nuclear height and roughness (R_q) were calculated and quantified for the corresponding nuclei. Surface roughness (R_q) was defined as root mean square average of height deviations.

Statistics and Reproducibility

For each experiment, sample sizes were chosen based on initial pilot experiments. Similar experiments reported in previous publications were further used to direct sample sizes. No data were excluded from the analysis. No blinding or randomization was used in the course of the experiments. All attempts of replication were successful. Statistical analyses were performed with Prism 7 (GraphPad). Data are presented as stated in the respective figure legends. All *t*-tests were performed as unpaired, two-sided *t*-tests.

Data availability

Mass spectrometry data have been deposited in ProteomeXchange with the primary accession code PXD213854. Datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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1 **Supplementary Information**

2 **1. Supplementary Figures**

3 **Supplementary Figure 1 | Nuclear actin levels during mitotic exit are not affected by**
4 **expression of nAC-GFP. Nuclear actin filaments are a conserved feature among different**
5 **mammalian cell lines and form independent of Emerin, Lamin A/C, and the LINC complex.**

6 **(a)** 3D nuclear fluorescence intensities (FI) of stably expressed mCherry-actin were
7 measured at indicated times after cell division (0 min corresponds to anaphase) to compare
8 its nuclear distribution in the absence or presence of co-expressed nAC-GFP. Nuclei were
9 counterstained with SiR-DNA. Data are shown as mean \pm SD (n=13 mitotic events per
10 condition, pooled from several independent experiments). **(b)** NIH3T3 cells stably
11 expressing mCherry-actin were transfected with GFP-Exportin 6 and followed during the
12 time-course of mitotic cell division. Images show single confocal slices to illustrate nuclear
13 distribution of mCherry-actin in postmitotic cells in either the presence (indicated by
14 asterisks) or absence (arrowheads) of GFP-Exportin 6. Note the nuclear fluorescence signal
15 produced by mCherry-actin in control daughter nuclei (indicated by arrowheads). The
16 experiment was performed once. Time stamp, hours:min; scale bar, 10 μ m. **(c)** Time-lapse
17 imaging of stably nAC-GFP (green) expressing MCF10A, HT1080, and RPE-1 during cell
18 division reveals nuclear F-actin formation at mitotic exit. Images show maximum intensity
19 projections. Scale bar, 10 μ m; time stamp, min:sec. **(d, e)** Quantification of incidence **(d)** and
20 duration **(e)** of nuclear F-actin formation during mitotic exit in MCF10A, HT1080, and RPE-1
21 cells stably expressing nAC-GFP. Data are shown as mean + SEM (n=40 (MCF10A, HT1080),
22 n=30 (RPE-1) mitotic events, pooled from several independent experiments). **(f, g)**
23 Quantification of the incidence **(f)** and duration **(g)** of nuclear F-actin formation during

mitotic exit in nAC-GFP expressing NIH3T3 cells, treated with indicated siRNAs. Data are shown as mean + SD (n=30 cells per condition, pooled from 2 independent experiments). **(h)** Immunoblot validating efficient siRNA-mediated knockdown of Lamin A/C and Emerin. The experiment was performed once. **(i)** Time-lapse imaging of NIH3T3 cells expressing nAC-GFP (grey) together with tagRFP-KASH (red, insets) to disrupt the LINC complex during and after mitotic division. The experiment was performed once. Scale bar, 10 μ m; time stamp, min:sec. Unprocessed original scans of blots are shown in Supplementary Fig. 7.

31

32

33 **Supplementary Figure 2 | Super-resolution imaging of nuclear F-actin at mitotic exit.**

34 **(a)** PALM images of NIH3T3 cells stably expressing nAC-Dendra2 (orange) at mitotic exit
35 used to calculate filament widths in Fig. 2b. Experimental resolutions after drift correction
36 (see Methods) are 38 ± 1 nm (1, 2, 4), 39 ± 1 nm (3), 33 ± 1 nm (5, 6), 35 ± 1 nm (7,8). Cells 5
37 and 6 are shown in Fig. 2a. Scale bars, 5 μ m. **(b)** Filaments were analysed by a self-written,
38 customized script for FIJI. First, ROIs were drawn along filaments (I). To minimize the
39 selection and pixelation error, selections were shifted by 0.5 pixels (5 nm) in all directions to
40 obtain five measures in total for each filament by straightening all selections (II). To
41 determine the filament width, lengthwise intensity profiles of the five filament selections
42 were fitted with Gaussians (III). The average full width at half maximum (FWHM) of the five
43 selections yields the average filament width. Scale bar, 0.5 μ m. **(c)** Mitotic cell division of
44 native NIH3T3 cells was monitored over time using DIC (Differential interference contrast)
45 to allow for formaldehyde fixation at defined time points after anaphase, as indicated.
46 Stochastic Optical Reconstruction Microscopy (STORM) images focus on the corresponding
47 nuclei labelled by phalloidin (see Methods for details). The dashed rectangle is shown

48 magnified in Fig. 2c. Experimental resolutions after drift correction are 40 ± 1 nm (15 min,
49 45 min-cell 1), 30 ± 1 nm (45 min-cell 2) and 34 ± 1 nm (60 min). Scale bars, 5 μ m.

50

51 **Supplementary Figure 3 | Actin dynamics but not transcriptional inhibition affect early G1**
52 **nuclear expansion. Detection of Flag-actin derivatives by doxycycline-inducible T2A-SNAP**
53 **fusions.**

54 **(a)** 3D surface reconstructions of NIH3T3 nuclei (visualized by SiR-DNA) at indicated times
55 after drug treatment at mitotic exit. Scale bar, 10 μ m; time stamp, hours:min. **(b)** Nuclear
56 volume quantifications in cells treated similar to **a**. Data is shown as mean \pm SD (n=50 nuclei
57 per condition, pooled from at least 3 independent experiments). **(c)** Design of Flag-tagged
58 nuclear actin derivatives linked to the SNAP-tag by a T2A peptide. Upon translation, the T2A
59 peptide is cleaved resulting in equimolar expression of Flag-NLS-actin and the SNAP-tag.
60 Accordingly, labelling of the SNAP-tag allows for indirect detection of Flag-NLS-actin in living
61 cells. **(d)** Immunoblot confirms doxycycline-induced expression of Flag-NLS-actin-T2A-SNAP
62 derivatives. A single band indicates efficient cleavage of Flag-NLS-actin-T2A-SNAP. **(e)**
63 Confocal images of fixed NIH3T3 cells expressing nAC-GFP transfected with Flag-NLS-actin-
64 T2A-SNAP derivatives. In contrast to Flag-NLS-actin the SNAP-tag (labelled by SiR-647)
65 displays pancellular distribution. Scale bar, 10 μ m. **(f)** Confocal images of fixed NIH3T3 cells
66 at mitotic exit show expression of Flag-NLS-actin-T2A-SNAP derivatives, as indicated.
67 Magnifications correspond to dashed rectangles and highlight Flag-actin. Scale bar, 10 μ m.
68 **(g)** Nuclear volume quantifications in live NIH3T3 cells stably expressing H2B-mCherry and
69 doxycycline-induced BFP-NLS, NLS-BFP-actin^{R62D} or Flag-NLS-actin-T2A-SNAP derivatives.
70 Expression of the indicated constructs was induced during G₀. Data are mean \pm SD from
71 n=30 nuclei per condition. **(h)** RT-qPCR analysis of *FOS* expression in serum-starved NIH3T3

72 cells, pre-treated with Flavopiridol (1 μ M for indicated times) before stimulation with serum
73 (20% FCS, fetal calf serum) for 30 minutes. Note that 15 min pre-treatment with Flavopiridol
74 efficiently inhibits serum-induced transcriptional upregulation of *FOS* mRNA. Data are
75 shown as mean from n=2 independent experiments. **(i)** Nuclear volume analysis in NIH3T3
76 cells stably expressing H2B-mCherry in the presence of Flavopiridol (1 μ M) or DMSO (0.1%).
77 Flavopiridol was added at metaphase prior to imaging the subsequent expansion of
78 daughter nuclei. Data are shown as mean \pm SD (n>14 nuclei [precise n?] per condition,
79 pooled from 3 independent experiments). Unprocessed original scans of blots are shown in
80 Supplementary Fig. 7.

81

82 **Supplementary Figure 4 | Inhibition of nuclear F-actin formation impairs chromatin**
83 **decompaction at mitotic exit**

84 **(a)** Quantification of GFP-H2B fluorescence lifetime in cells expressing GFP-H2B alone, or in
85 combination with mCherry-H2B and indicated treatments: trichostatin A (TSA), or sodium
86 azide (NaN₃) together with 2-deoxyglucose (2-DG). ***P* < 0.01, ****P* < 0.001 calculated by
87 one-way ANOVA. **(b)** Quantification of fluorescence lifetime reveals no significant difference
88 in GFP fluorescence lifetime upon expression of and staining for Flag-Exportin 6 using a
89 Alexa Fluor 405-conjugated antibodies. ns, non-significant in one-way ANOVA. **(c)**
90 Comparative immunoblot analysis of histone modifications (H3S10ph, H4K16ac) in NIH3T3
91 cells induced to express BFP-NLS or NLS-BFP-actin^{R62D} and undergoing either asynchronous
92 (asyn.) or synchronized (mitotic shake off) culture, as indicated. **(d, e)** Images and
93 quantitative immunofluorescence analysis of nuclear Aurora B **(d)** and KAT5 **(e)** (both green;
94 nuclei are stained with DAPI (magenta)) in NIH3T3 cells at mitotic exit expressing Flag-NLS-
95 actin-T2A-SNAP derivatives, as indicated. Data are shown as mean \pm SD (n=20 nuclei per

condition, pooled from three independent experiments). Scale bar, 10 μm . **** $P < 0.0001$ calculated by t -test. **(f)** Analysis of chromatin compaction by an MNase accessibility assay 45 min after mitotic shake-off in NIH3T3 cells expressing either doxycycline-induced Flag-NLS-actin^{wt} or -actin^{R62D}. Graph shows quantified pixel intensities corresponding to band intensities. **(g)** Example images illustrating the pipeline used for the quantification of condensed chromatin in cryo-electron microscopy images (for details see Methods). Based on raw images (I) nuclei and nucleoli were manually segmented (II). Condensed chromatin was semi-automatically segmented across the nucleoplasmic region and classified (III) allowing for an assessment of its distribution using a custom ImageJ/Fiji macro (IV). **(h)** Representative electron microscopy images of cryo-preserved, synchronized early G₁ NIH3T3 cells expressing GFP-Exportin 6 corresponding to Figure 4I. Scale bar, 2 μm . Unprocessed original scans of blots are shown in Supplementary Fig. 7. Immunoblot in c and MNase accessibility assay in f represent 1 out of 2 independent experiments.

Supplementary Figure 5 | Inhibition of nuclear F-actin formation impairs Pol II-dependent transcription and preimplantation development of mouse embryos.

(a) Images corresponding to Figure 5a showing RNA Pol II pS5 stainings (grey) in NIH3T3 cells expressing either GFP or GFP-Exportin 6 at indicated times after mitotic shake-off. Scale bar, 5 μm . **(b)** Images corresponding to Figure 5g showing preimplantation development of mouse embryos expressing either mCherry-Exportin 6 or myc-tagged GFP as a control. Similar amounts of mRNA were injected into oocytes at the metaphase II stage, followed by *in vitro* fertilization. Scale bar, 100 μm .

Supplementary Figure 6 | P-Cofilin levels change during mitotic exit and nuclear Cofilin-1 is essential for filament disassembly during mitotic exit.

(a) Representative immunostaining of p-Cofilin (grey, DAPI (blue)) in NIH3T3 cells treated with si-Control or si-Cofilin to validate specificity of the obtained fluorescence signals. Asterisks indicate presumably non-silenced cells. Scale bar, 10 μ m. The experiment was performed once. **(b)** Images corresponding to quantifications shown in Figure 6e. Confocal images show single slices at indicated time points after mitotic shake-off. Scale bar, 5 μ m. **(c)** Immunoblot detecting p-Cofilin and Cofilin in RPE-1 cells after washout of nocodazole. Decreasing H3S10ph levels proof for successful release from the nocodazole-induced mitotic block. **(d)** P-Cofilin/Cofilin ratio was calculated by densitometric quantification of immunoblot intensities. Data are shown as mean + SD from n=3 independent experiments. **(e)** Time-lapse imaging of NIH3T3 cells during mitotic exit corresponding to Figure 6j. Cells stably express nAC-GFP (green) together with either WT- or NES-mCherry-Cofilin (red) and were treated with siRNA against the 3'-UTR of endogenous Cofilin-1. Scale bar, 10 μ m. **(f)** Stably nAC-GFP expressing NIH3T3 cells were transfected with NLS-mCherry-Cofilin and followed during mitotic exit. Images show maximum intensity projections of confocal z-stacks and illustrate the absence of nuclear F-actin formation which was observed in 10 of 12 mitotic events. Time, hours:min; scale bar, 10 μ m. **(g)** Immunoblot validating expression of opto-Cofilin in cells treated with either control siRNA or siRNA directed against the 3'-UTR of endogenous Cofilin-1. Unprocessed original scans of blots are shown in Supplementary Fig. 7.

Supplementary Figure 7 | Unprocessed original scans of Western blot analysis

2. Supplementary Tables

Supplementary Table 1

Table illustrating the incidence and duration of nuclear F-actin formation at mitotic exit upon siRNA-mediated knockdown of actin nucleators or regulators. All siRNA sequences used showed greater than 50% knockdown efficiency on mRNA level for the intended target, as determined by RT-qPCR (compared to control siRNA and normalized to expression of TBP). Statistical analysis did not show a significant difference for any condition compared to control cells, as determined by one-way ANOVA. n.d., not determined. Data are shown as mean +/- SEM, pooled from at least 2 independent experiments. Sample sizes (number of mitotic events) for each condition are shown within the table.

Supplementary Table 2

Table summarizing the results of nuclear F-actin pulldown, as well as a control pulldown (without biotin-phalloidin), performed at mitotic exit and analysed by mass spectrometry (see Methods for details).

Supplementary Table 3

Table listing the information of antibodies used in this study.

3. Supplementary Videos

Supplementary Video 1 | Transient nuclear F-actin formation can be detected during mitotic exit.

Video corresponding to Fig. 1a shows transient formation of nuclear F-actin during and after cell division in NIH3T3 cells as visualized by nAC-GFP (green). In addition, cells express LifeAct-mCherry (red). Scale bar, 10 μ m.

Supplementary Video 2 | Nuclear F-actin shows dynamic turnover in cells at mitotic exit.

Video corresponding to Fig. 1b shows dynamic reorganization of actin filaments after mitotic division in NIH3T3 cells as visualized by nAC-GFP (green). In addition, cells express Lamin-nanobody-SNAP, labelled by a SiR-647 dye (LaminCB-SNAP|SiR-647, magenta). Scale bar, 10 μ m.

Supplementary Video 3 | Nuclear F-actin forms within interchromatin spaces.

Video corresponding to Fig. 4a shows dynamic reorganization of actin filaments after mitotic division in NIH3T3 cells as visualized by sAC-GFP (green). In addition, cells express H2B-mCherry (red) to visualize chromatin content. Scale bar, 10 μ m; time stamp, h:min:s.

Supplementary Video 4 | Nuclear actin filaments reshape newly assembled nuclei.

Video corresponding to Fig. 3a shows NIH3T3 cells during mitotic exit, stably expressing nAC-GFP (green) and H2B-mCherry (red). Scale bar, 10 μ m; time stamp, min:s.

Supplementary Video 5 | Knockdown of Cofilin affects nuclear actin dynamics during mitotic exit.

Video corresponding to Fig. 6f, g. Time-lapse imaging of NIH3T3 cells stably expressing nAC-GFP (green), treated with si-control or si-Cofilin during mitotic exit. Video shows three representative examples for each condition. Note the appearance of excessive and stable nuclear actin filaments in si-Cofilin-treated cells. Scale bar, 10 μ m.

Supplementary Video 6 | Light-regulated control of opto-Cofilin subcellular localization.

Video corresponding to Fig. 7d shows NIH3T3 cells stably expressing opto-Cofilin (grey). Single confocal slices were acquired at 10 sec intervals, and cells were temporarily illuminated by additional blue laser light (488 nm, indicated by a green bar) to promote reversible nuclear export of opto-Cofilin.

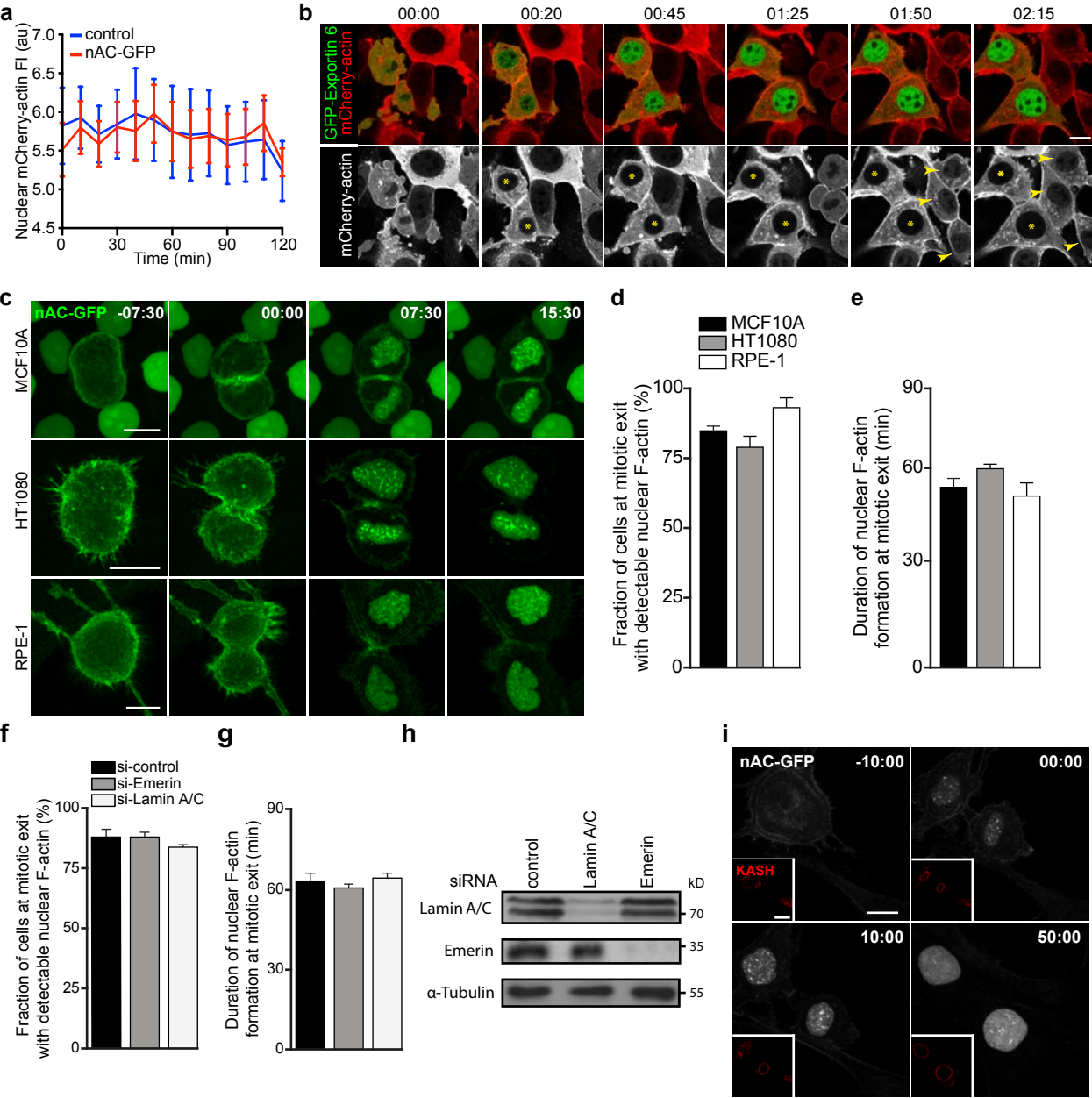
Supplementary Video 7 | Formation of excessive, stable nuclear F-actin upon light-regulated nuclear exclusion of opto-Cofilin.

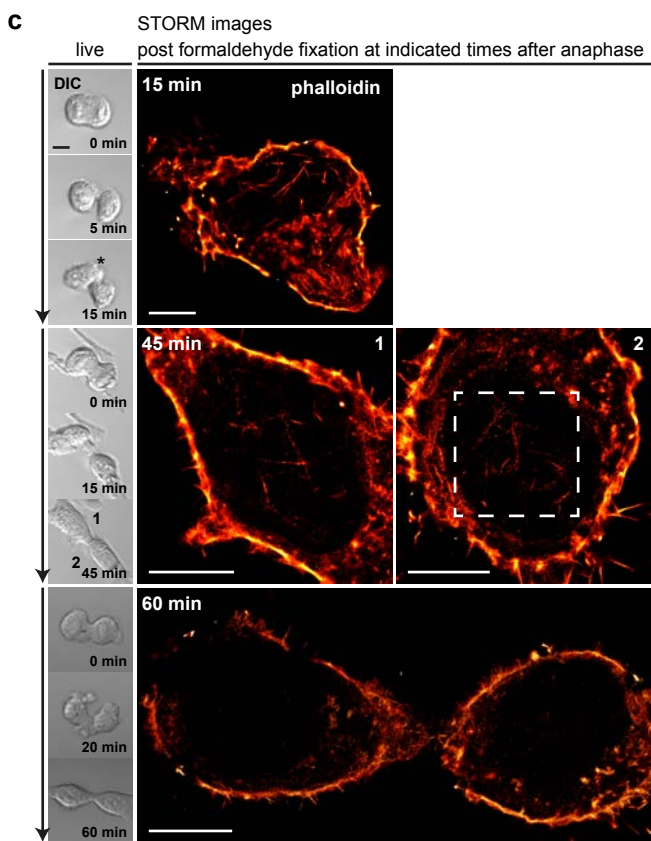
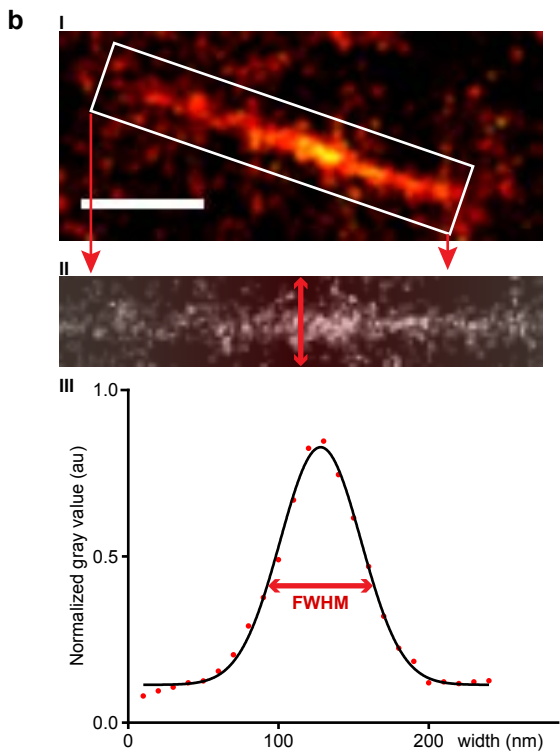
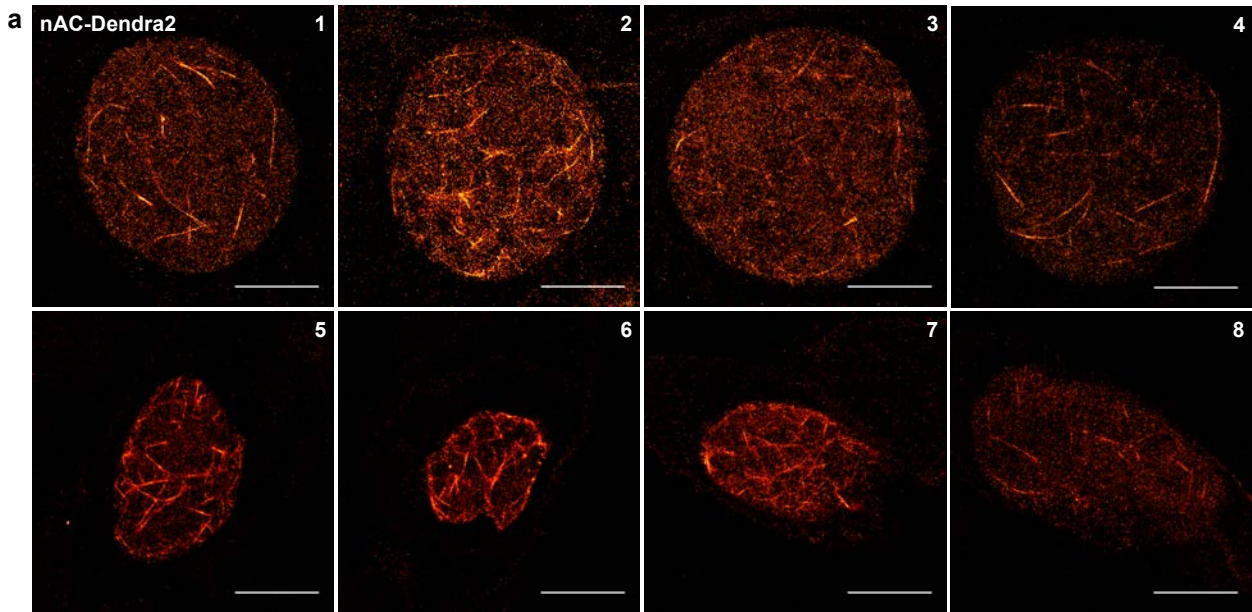
NIH3T3 cells stably expressing nAC-SNAP (labelled by SiR-647, grey) and opto-Cofilin (red) were treated with si-Cofilin (3'-UTR) and imaged during and after mitosis. Cells were imaged either with (+ light, lower panel) or without (- light, upper panel) additional blue laser light (488 nm) to promote sustained nuclear export of opto-Cofilin.

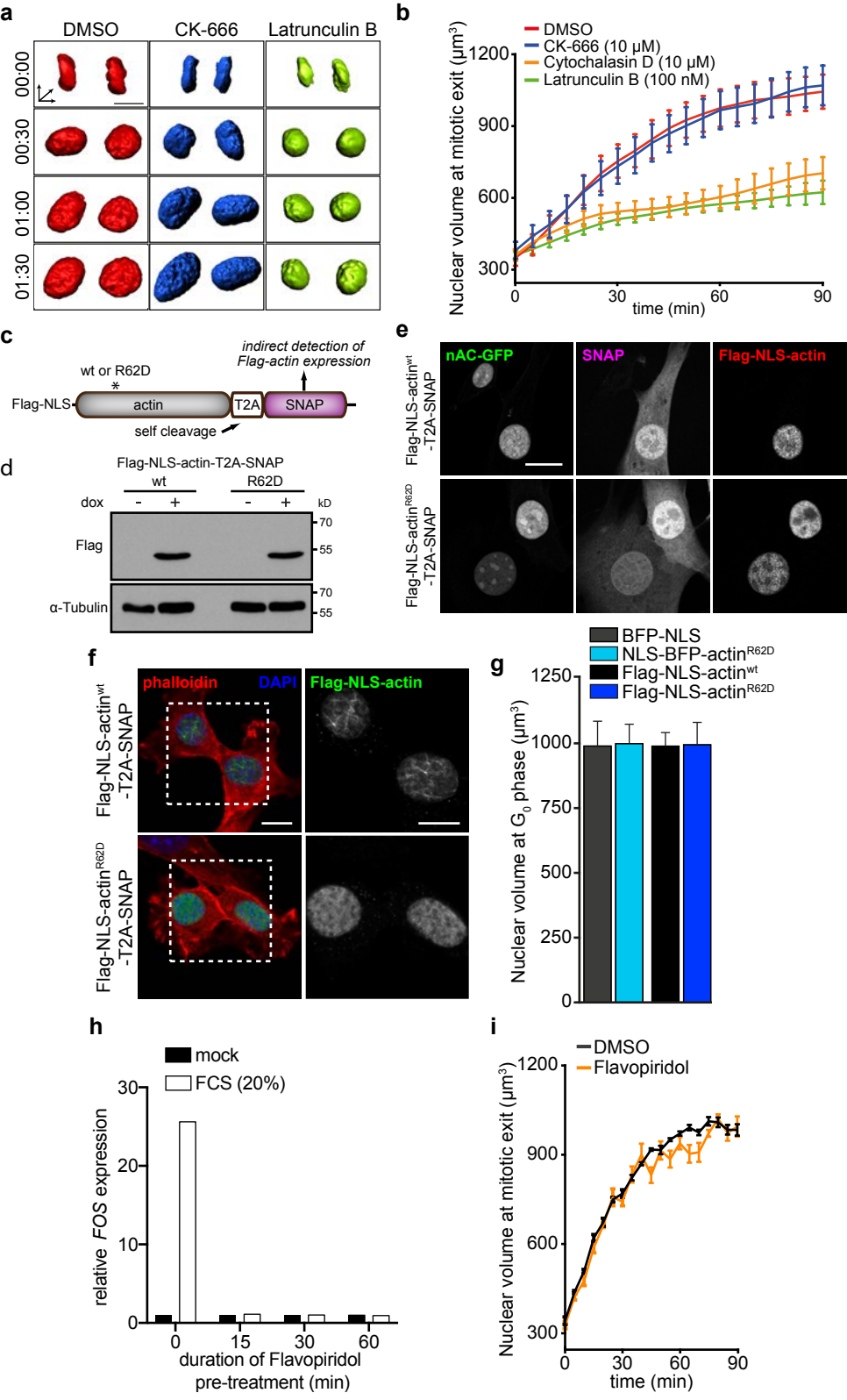
Supplementary Video 8 | Reversible formation of excessive, stable nuclear F-actin by light-controlled subcellular shuttling of opto-Cofilin.

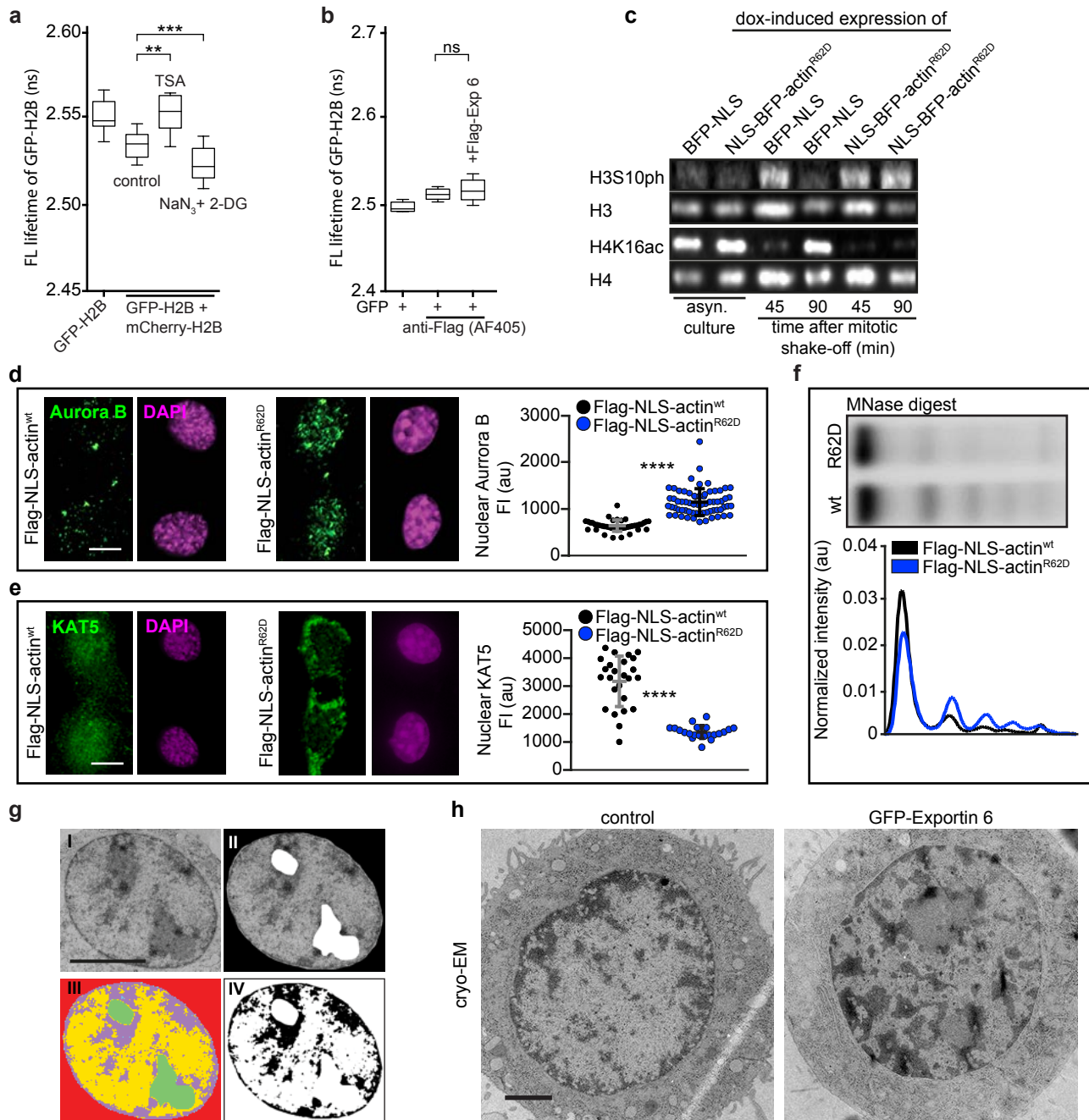
Video corresponding to Fig. 7f shows NIH3T3 cells stably expressing nAC-SNAP (labelled by SiR-647, grey) and opto-Cofilin (red) during and after mitosis. Cells were treated with si-

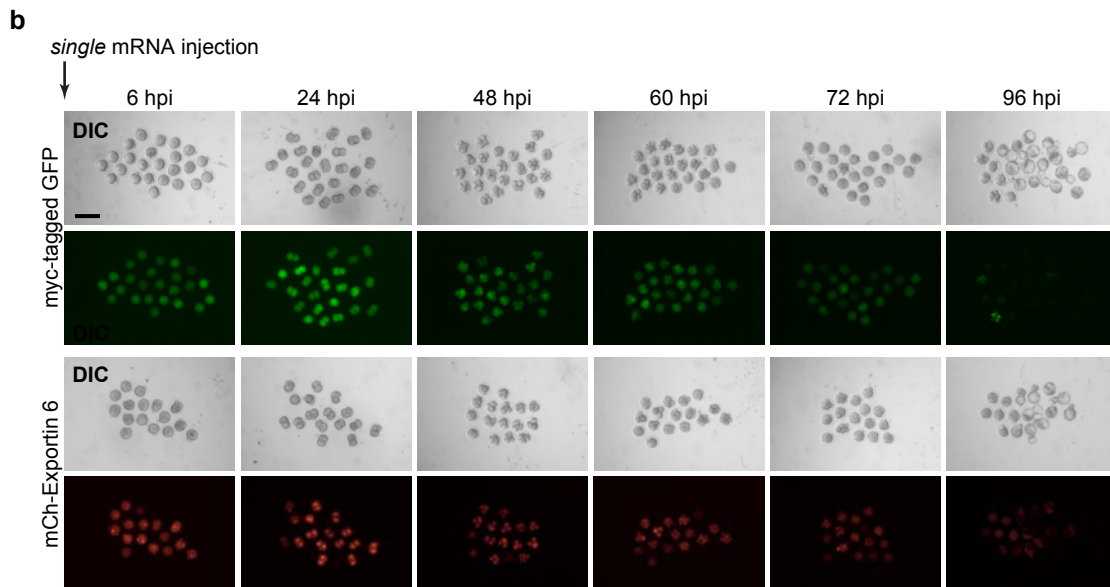
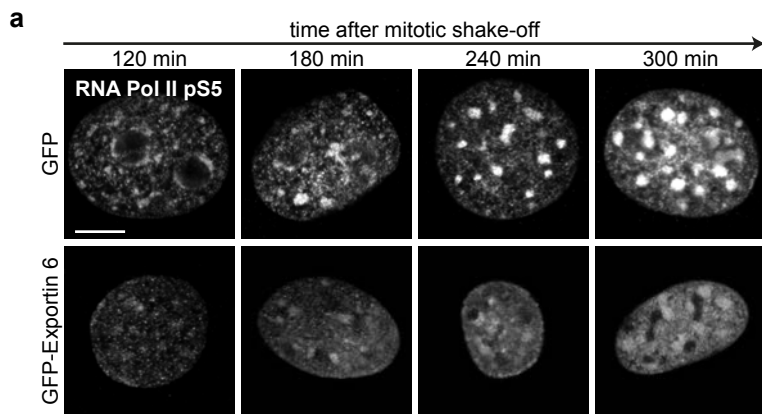
210 Cofilin (3'-UTR) and temporarily illuminated by blue laser light (488 nm) to promote nuclear
211 export of opto-Cofilin for a defined period of time (indicated by a green bar).
212











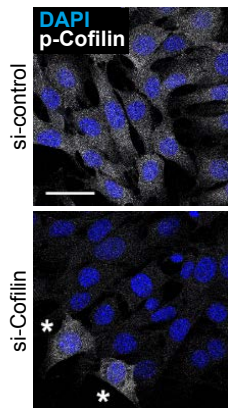
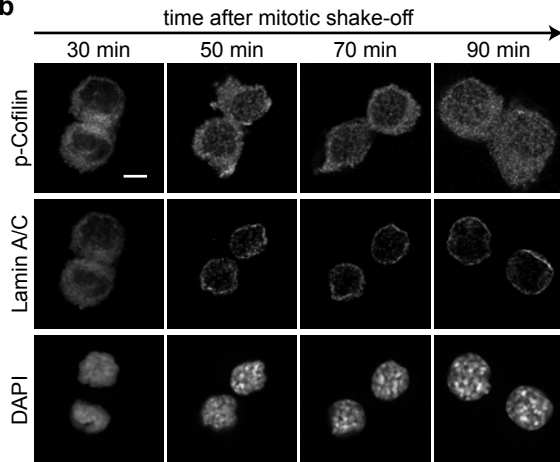
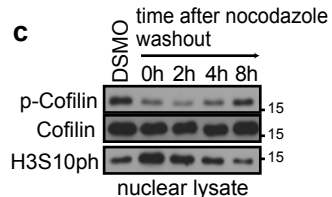
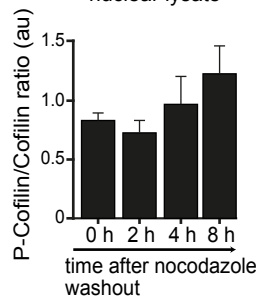
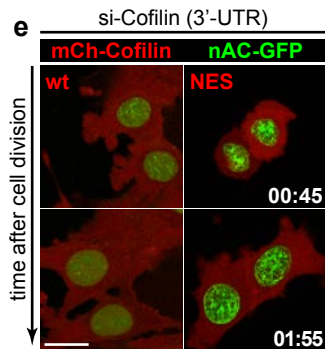
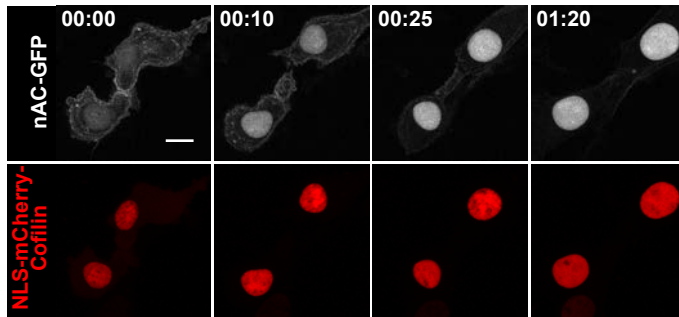
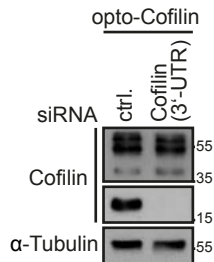
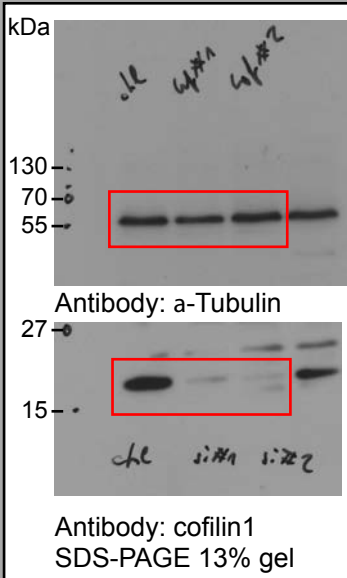
a**b****c****d****e****f****g**

Figure 6g



Suppl. Figure 3d

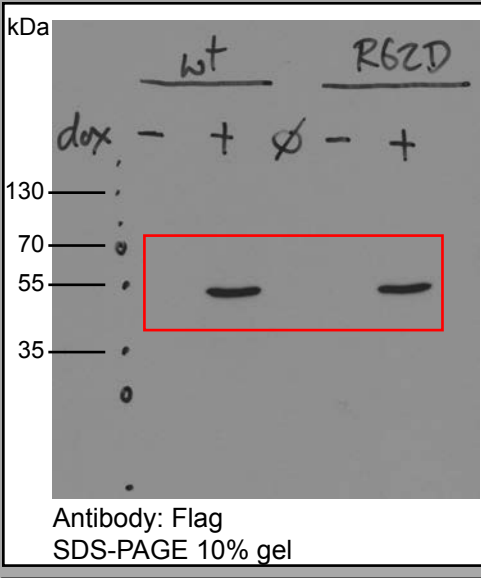
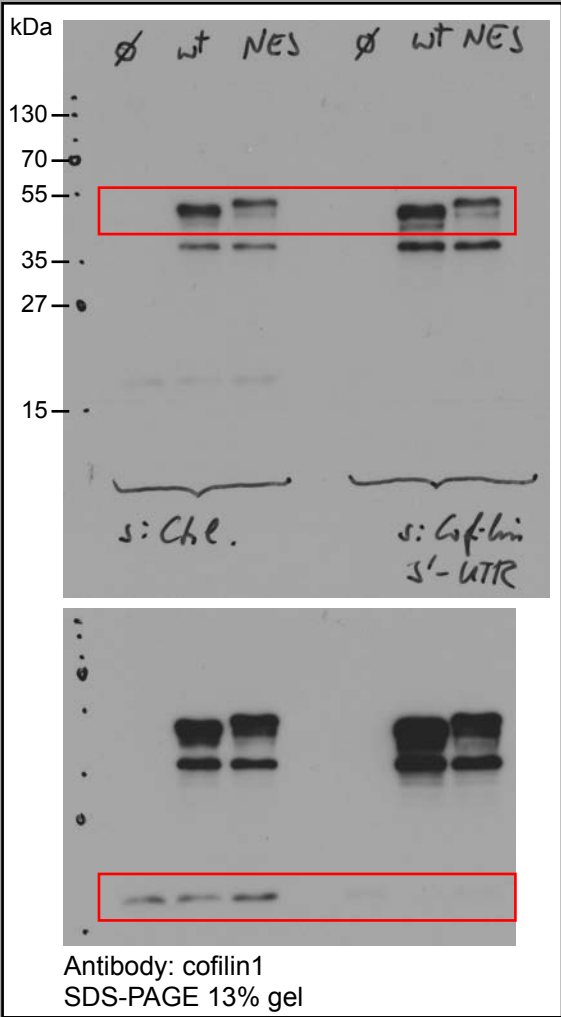
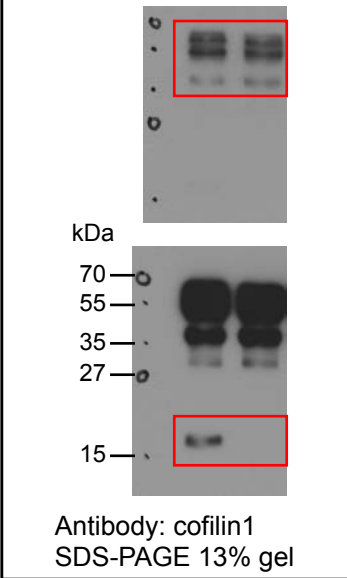


Figure 6i



Suppl. Figure 6h



Suppl. Figure 6h

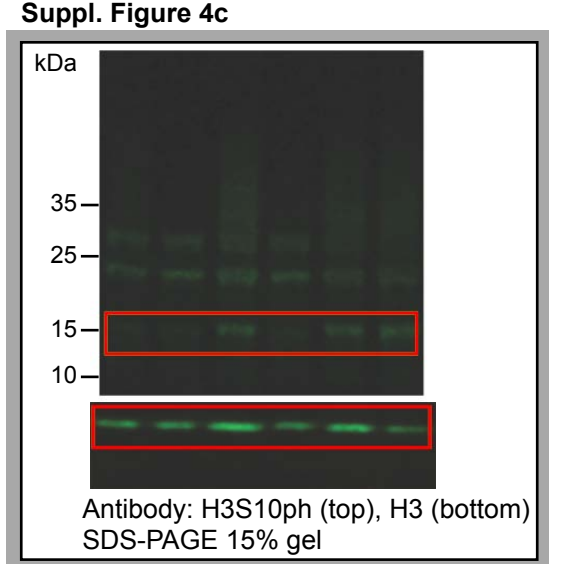
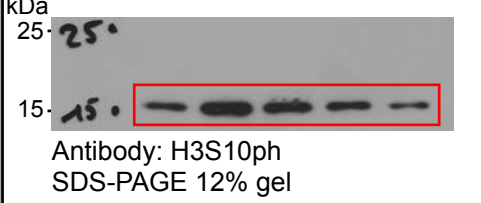
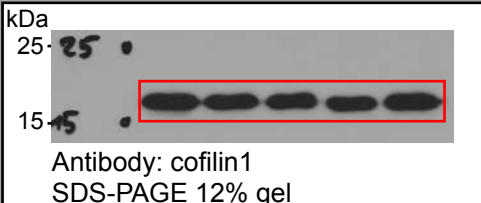
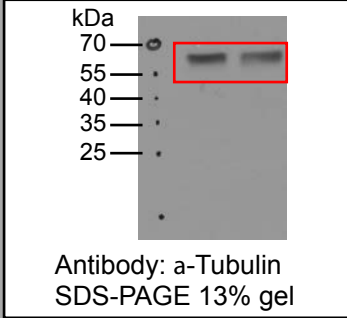
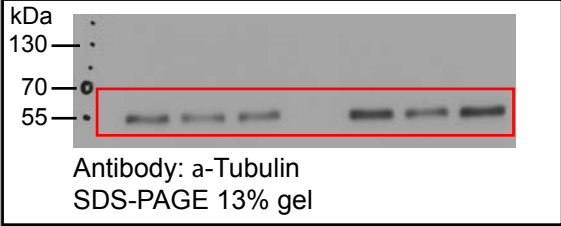
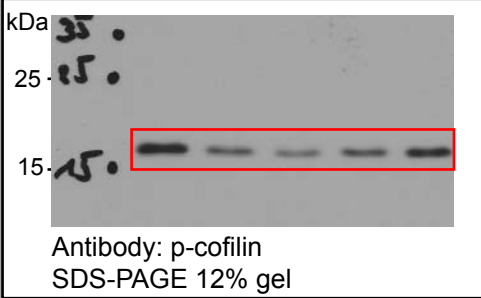
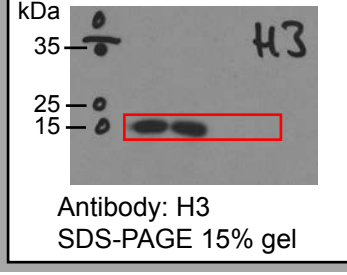
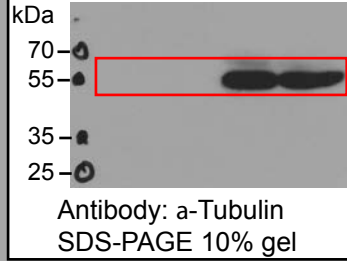


Figure 6b



Suppl. Figure 1h

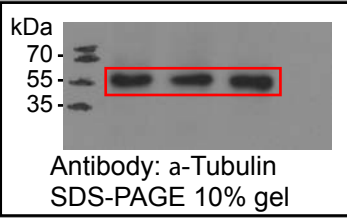
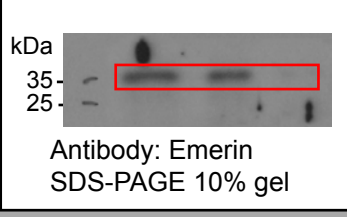
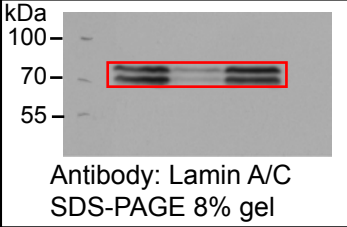


Figure 6c

